



Pioneering Innovations in Biological Weed Control: A Comprehensive Review

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ABSTRACT

The biological method of weed control is an effective option. Before implementing bio-agents, there is a need for a better understanding of the mechanisms involved in interactions by multiple agents against weeds. This covers knowledge of how plants integrate and prioritize their responses to multiple attackers. Across the globe, terrestrial ecosystems are known to be characterized by a huge diversity of species and a corresponding diversity of interactions between these species. These include species of different trophic levels of habitats on the same food chain. Plants undoubtedly play significant role as intermediaries in interactions between the insect and microbial populations with which they are involved. Plant interactions brought forth by one species can have cascading impacts on other species, influencing their abundances and the composition of communities. Several research efforts are underway to develop highly effective biological control agents to reliably control a problematic broad spectrum of weeds. Sustainable and ecosystem-compatible weed management for controlling weeds by biological means, such as insects, bio-agents, myco-herbicides and harmful Rhizobacteria. Their interaction with cultural practices and allelochemicals are some biological components of sustainable agriculture. Hence, biological agents enhance efficiency and restrict the detrimental effect of weeds compared to weeds being left alone. The challenge is to identify appropriate microbe/bioagents, which reduce weed growth. Logically, biological weed management is the only way to control weeds in eco-friendly weed management.

Key words: Biocontrol agents, Bioherbicides, Biological weed control, Harmful rizobacteria, Insect agents, Microbial interactions.

Biological control of weeds by using plant pathogens has gained acceptance as a practically safe, environmentally beneficial, weed management method applicable to agroecosystems. When the environment, the biocontrol agent and the weed interact in a way that effectively suppresses or controls the weed, biological control is successful. Biological control often means the introduction of organisms into an ecosystem to control one or more undesirable species (Vikas *et al.*, 2016). It is an important tool for integrated weed management. Before releasing bio-agents, we should fully understand the mechanisms involved in interactions by multiple agents against weeds. By 1925, Australia was struggling with 60 million acres of grazing land heavily infested with prickly pear cactus. Hundreds of square miles were virtually impenetrable to humans or animals. It was like a miracle that a small moth from Argentina (*Cactoblastis cactorum*) was imported and released, which impacted the moth larvae burrowed into the cactus, grew and multiplied. Within 10 years it reduced the prickly pear population. Today, the cactus covers only 1% of the area it occupied in 1925.

Insects that are above ground, such as herbivores, pollinators and their natural enemies, interact with the helpful underground bacteria throughout the plant in both directions. The significance of these relationships for agricultural and natural ecosystems is growing and the physiological and molecular mechanisms underpinning these links deserve further research. The beneficial interactions between above-ground insects and below-

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ground microbes mediated by plants are reviewed here. Pollinators, natural enemies and herbivorous insects are all impacted by changes that various soil-borne microbes cause in the plant. Herbivores' performance is impacted by the beneficial microbes because they promote plant growth and create resistance in aerial plant tissues. Beneficial insects are primarily attracted to plants that emit volatile organic compounds (VOCs).

An enormous diversity of species and an equally diverse range of interactions between species, involving species at different trophic levels within the same food chain are characteristics of terrestrial ecosystems (Arjen and Alison, 2013). Insects and microbial communities interact with each other through plants, which act as crucial mediators. Plant interactions with other species are frequently influenced by changes brought about by one species, which shape the overall structure and abundance of the communities. The release of multiple agents against weeds has proven to be more successful than the release of a single agent, as demonstrated by many recent studies (Chavan *et al.*, 2021).

The success of the Argentine cactus moth in controlling the invasive prickly pear in Australia in the 1920s was partly based on its feeding wounds which provided access to secondary pathogens that killed the cactus (Caesar, 2000). This study highlights the scope of multitrophic interactions in biological control programmes, particularly where the agents are known to be susceptible to parasitism.

It frequently happens that the release and establishment of numerous agents with cumulative effects is necessary for an effective biological control campaign against persistent weeds (Syrett *et al.*, 2000). In nature, interactions between several natural enemy species and their hosts are frequent since they may be targeting the same portion of the plant (Kruess, 2002). Plant performance may be affected by these interactions in a synergistic (Buccellato *et al.*, 2012) or inhibitory (Hatcher and Paul, 2002) way. It is challenging to foresee how these interactions may affect the host plants, though. For instance, a phytopathogen may cause a chemical change in the host plant that causes the herbivore to eat the diseased tissue (Hatcher *et al.*, 2004). On the other hand, fungal infection can cause the build-up of large concentrations of defensive chemicals, which might decrease the palatability of plant tissues (Karban *et al.*, 1987; Piening, 1972). The effectiveness of biological weed control mostly depends on the degree to which herbivores and phytopathogenic fungi influence one another, as mixtures of natural enemies are frequently employed in this process. However, there aren't many studies that measure how pathogen and insect biocontrol agents interact when they simultaneously take advantage of weeds. Nonetheless, the significance of interactions between herbivores, plants and diseases has been recognized (Caesar, 1996; Caesar 2000), with multiple writers highlighting the necessity of integrating pathogens and insects (Caesar, 2011).

Invading species may often escape their herbivores and pathogens during the invasion process, invasive plant species enhance their performance in the non-native plant species by increasing the abundance of pathogens which are adverse to native plants, plants can either benefit or suppress mutualistic from invasive plants. Multitrophic interactions tend to refer to the complex relationships and interactions that occur among multiple trophic levels within an ecosystem. In ecology, trophic levels represent different

levels in the food chain, where each level consists of organisms that share similar feeding relationships. A food chain typically includes producers (plants), primary consumers (herbivores), secondary consumers (predators) and so on. Multitrophic interactions mostly go beyond simple predator-prey relationships and involve the interplay of various organisms, including plants, herbivores, predators, parasitoids and decomposers (Caesar, 2003; Caesar, 2013). These interactions can be direct or indirect and may have significant impacts on ecosystem dynamics, species coexistence and functioning of the ecosystem (Fowler *et al.*, 2000).

For example, in biological weed control, there is potential for multitrophic interactions between weeds, herbivorous insects and pathogens that target the weeds, the natural enemies of these herbivores and even the influence of neighbouring plants or soil microbes on weed suppression. The improved understanding and harnessing of these complex interactions is key to developing innovative and sustainable approaches to weed management. By studying multitrophic interactions, researchers can uncover new perceptions of the natural dynamics of weed control and so also explore novel strategies for biological weed management. This knowledge can lead to the development of innovative ideas and techniques that leverage intricate web of relationships within ecosystems to effectively control weeds, reducing the reliance on synthetic herbicides and promoting sustainable agricultural practices (Kashyap *et al.*, 2022).

Plant as a centre of Multitrophic Interactions

Plants exist along with biological competitors that exert various degrees of top-down regulation of host populations. Individual plants often respond differently to alterations in competitive background like plants inoculated with viruses during feeding by insects versus by other vectors (insects/pests, water, air, humans *etc.*) (Fig 1). Some studies (Ray and Sushil, 2009) have pointed out that some herbivory may result in overcompensation and may benefit the host plant. One of the important mechanisms underlying possibilities

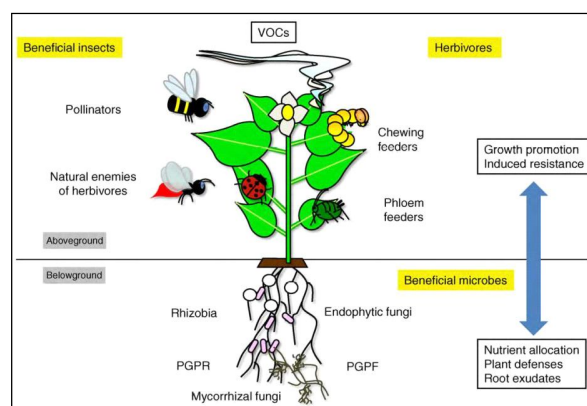


Fig 1: Plant as a centre of multitrophic interactions.
(Pineda *et al.*, 2012).

of multitrophic interaction is the induction of plant defences by insects and beneficial or pathogenic microbes that result in cross-resistance or susceptibility. Others (Ray and Hill, 2016) have indicated that plants are often detrimentally affected by defoliation and respond by undercompensation. Still other studies (Maschinski and Whitham, 1989) have shown that herbivores may have little effect on the plants on which they feed. Then biocontrol agents may have a positive or negative impact on each other to the plants (Ray and Hill, 2015).

Possibilities of multitrophic interactions in biological weed control (BWC)

Possibilities of multitrophic interactions (PMI) should be studied as an essential part of pre-release assessments to enhance success rates of biological control of weeds. Studies by (Ray and Sushil, 2009) have pointed out that some level of herbivory may result in over-compensation and may benefit the host plant. One underlying PMI interaction is the induction of plant defences by insects and beneficial or pathogenic microbes that result in cross-resistance or susceptibility. Ray and Hill (2016) have indicated that plants are detrimentally affected by defoliation and respond under compensation. Still, other studies (Maschinski and Whitham, 1989) have shown that herbivores may have little effect on plants on which they feed. Then biocontrol agents may have a positive or negative impact on each other to the plants (Ray and Hill, 2015).

The co-existence of the arthropod and fungal biological control agents can be both beneficial and detrimental to each other depending on their interactions with the host plant. Insect-feeding wounds may provide entry for the fungi (Lane and Gladders, 2000). Infection by fungi or insect herbivory may induce changes in plant metabolites which may in turn positively or negatively influence insect living and the disease-causing potential of the pathogen (Julien and Griffiths, 1998). Insects may deliver fungal spores on the cuticular surface by its appendages or digestive excreta (Studhalter and Ruggles, 2009). The biological approach (deliberate use of natural enemies to suppress the growth or reduce the population of the weed species of managing weeds is gaining momentum (Sushil and Ray, 2008; Sushil, 2009).

Impact of interactions within pairs of insects (bio-agents) on weed control

Form a toxic compound upon rupture of multiple cells by chewing, defoliation, oozing fluids, scorching, shot holes and mining (Smith and Hallett, 2006). Both the larvae and adults feed on grasses and their life cycle is primarily completed inside or outside plants (Philip *et al.*, 1981). Insects attack seeds and reduce the number of weed seeds stored in the soil, which in turn reduces the size of the next season's weed populations. Insects may deliver fungal spores on cuticular surfaces by their appendages or digestive excreta (Studhalter and Ruggles, 2009). Water hyacinth, *Eichhornia crassipes*, was the subject of studies on interactions between pairs of

biological control agents (Daniels Alicia *et al.*, 2023). The findings indicated that following an 11-week exposure to herbivorous combinations of three agents - mite, *Orthogalumna terebrantis*; weevil, *Neochetina eichhorniae* and mirid, *Eccritotarsus catarinensis* - the water hyacinth leaf surface area was damaged on leaves (Singh *et al.*, 2015). When the water hyacinth leaf 5 areas were damaged and subjected to herbivory after 11 weeks using various combinations of the three agents (mite *Orthogalumna terebrantis*, weevil *Neochetina eichhorniae* and mired *Eccritotarsus catarinensis*) (Danica *et al.*, 2013).

Impact of microbes (bio-herbicides) interactions on weed control

The impact of the efficacy of spore suspensions and cell-free metabolites against weeds is often manifest as cell-free metabolites of the fungi were also sprayed or in combination (Salim and Naseema, 2005) which include pathogens high in the production of enzymes like cellulose, pectinase and proteinase (Fig 2). Induced systemic resistance induced by phenol, polyphenol oxidase and catalase could be impacted by the formulation of *F. pallidoroseum*-effective mycoherbicide for the management of water hyacinth and develop blight symptom on the 4th day and recorded 95% damage by 7th day (Singh *et al.*, 2013). To susceptible and resistant populations it showed differential effects on growth and emergence of weed which need further verification (Bhagirath, 2012).

Harmful *Rhizobacteria*: The rhizobacterial approach to biological weed control

The traditional biological control of noxious weeds involves the use of fungi and insects. Since the 1970s, successful mycoherbicide development has been achieved for particular weeds in restricted areas. Yet, harmful rhizobacteria, which are defined as non-parasitic bacteria (exopathogens) that colonize plant root surfaces and can inhibit plant growth, are

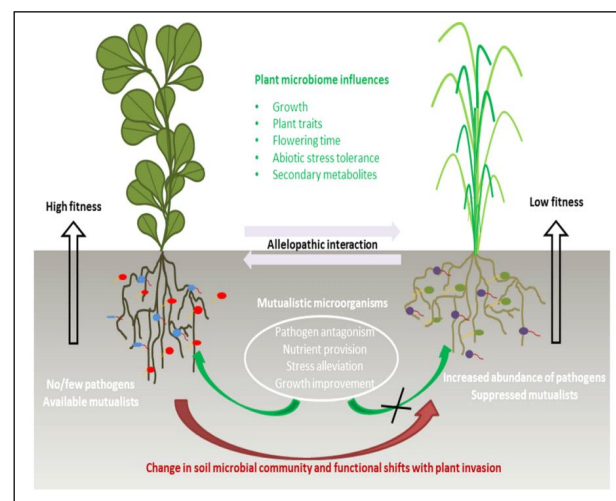


Fig 2: Schematic overview of microbial interactions between weeds/invasive plants and crop/native plants.

Table 1: *Rhizobacteria* associated with weed-crop ecosystems.

<i>Rhizobacteria</i>	Weeds	Ecosystems
<i>Enterobacter</i>	<i>Bromus tectorum</i>	Cereals
<i>Enterobacter</i>	<i>Amaranthus sp.</i>	Row crops
<i>Pseudomonas</i>	<i>Setaria viridis</i>	Cereals and range lands
<i>Pseudomonas</i>	<i>Calamagrostis canadensis</i> var <i>Canadensis</i>	Forest nursery
<i>Pseudomonas</i>	<i>Bromus japonicus</i>	Cereals
<i>Pseudomonas</i>	<i>Abutilon theophrasti</i>	Cereals
<i>Pseudomonas fluorescens</i>	<i>Aegilops cylindrica</i> , <i>Sisymbrium amaranthus</i>	Cereals
<i>Xanthomonas</i>	<i>Bromussecanus</i> , <i>Aegilops cylindrica</i>	Cereals
<i>Typhlodendron flavobacterium</i>	<i>Euphorbia esula</i>	Range lands
<i>Fraxinibacter</i>	<i>Setaria sp</i>	Row crops

the microbes that are frequently disregarded as biological control agents of weeds (Table 1) harmful rhizobacteria typically colonize the rhizosphere of specific weeds and inhibit the growth and multiplication of their roots. Weeds continue to be stunted. As a result, it difficult for crop plants to compete with them. harmful rhizobacteria is a modern strategy that requires in-depth fieldwork. Harmful rhizobacteria may be important traditional bacterial pathogens that have an impact on plant growth despite their non-parasitic, subtle mode of attack (Venter *et al.*, 2012). Harmful rhizobacterias are classified as exopathogens or non-parasitic bacteria, that colonize plant root surfaces and can inhibit plant growth. Certain weeds usually have rhizobacteria colonize their rhizosphere, inhibiting the growth and multiplication of their roots harmful rhizobacteria may have an equal impact on plant growth as conventional bacterial pathogens, despite its non-parasitic subtle attack mode (Suslow *et al.*, 1982). Also, many harmful rhizobacteria are plant specific (Schroth and Hancock, 1982) and this could open up a possibility that the existence of harmful rhizobacteria on weeds could be exploited as a biological control, which further assumes to be an important tool/ component for effective weed management in the integrated weed management system. This has geared up research globally on deleterious rhizobacteria in recent years, while harmful rhizobacteria were first described on downy brome (*Bromus tectorum* L.) occurring in winter wheat fields (Cherrington and Elliott, 1987).

To generate a high population of bacteria in the rhizosphere and to obtain a rapid beginning of growth inhibitory activity, the practical application of harmful rhizobacteria for weed management entails the employment of an inundative method for delivering inocula. Traditionally, harmful rhizobacteria have been applied directly to the soil or vegetative wastes in field trials to attack germination seeds and emerge seedlings ultimately suppressing weed development. Controlling weeds either before or concurrently with crop plant establishment is the rhizobacteria method. Consequently, harmful rhizobacteria does not always mean eliminating problematic weeds; rather, it greatly inhibits weeds' early growth and makes it possible for emerging crop plants to successfully compete with the weaker weed seedlings for growth requirements.

It appears that harmful rhizobacteria works better when weed development occurs in conjunction with conditions that support bacterial growth and plant suppression (Johnson *et al.*, 1996). Harmful rhizobacteria mainly work by producing a phytotoxin that is absorbed by the roots of seedlings (Tranel *et al.*, 1993). The effectiveness and host specificity of rhizobacteria are related to their application.

Herbicides are more successful than harmful rhizobacteria as bio-control agents since the former works mainly by suppressing growth. According to field experiments, crop yields in plots with inhibited weed development were substantially higher than those in plots with thriving weeds. Under field conditions, *Pseudomonas sp.* isolated from the same brome roots is shown to biologically suppress downy brome in winter wheat (Kennedy *et al.*, 1991) The bacteria usually have no effect on winter wheat density, but they greatly enhance grain yield. This was mainly because the bacteria had a growth-suppressive effect on downy brome, which made wheat more competitive. Additionally, as several other trials have demonstrated, harmful rhizobacteria dramatically decreased downy brome seed production. At least eleven projects in the United States and Canada are actively working to develop harmful rhizobacteria for weed biological control. The harmful rhizobacteria have designated 18 weed species as targets for control. Most of the projects concentrated on managing annual weeds which are significant economic factors in cereals and row crops. *Pseudomonas sp.* has been identified in almost all projects as the main group with the potential to be successful candidates for biological control. With less of an inhibitory effect on crop seedlings, the cyanogenic strain *P. aeruginosa* KC1 appears to have the deleterious ability to suppress weed seedling growth, both in vitro and under glasshouse conditions. This presents an opportunity for the development of efficient systems based on bacteria that can be integrated with biological weed control management (Vijaya *et al.*, 2015).

Synergistic interaction between synthetic and microbial herbicides for weed control

Many herbicides impair the ability of weeds to respond to pathogen attacks (Ahn *et al.*, 2005). Additional benefits include a broadened spectrum of weed control and a wider

window of application under field conditions. Herbicides tend to weaken the weeds and impair their defence systems, often making weeds more vulnerable to mycoherbicide infection. Glyphosate suppressed the biosynthesis of a phenylpropanoid phytoalexin to kill sickle pod, due to prior application of *Alternaria cassia* and this inhibition occurred even at reduced rates of glyphosate (sub lethal dose). There is great need for fine tuning doses to maximize efficacy in field conditions, while herbicides should be applied prior to mycoherbicide agents (Smith and Hallett, 2006) and similar results are found with the effect of *Pyricularia setariae* (5×10^7 spores/ml) plus sethoxydim on green foxtail in greenhouse and field condition (Gary and Thomas, 2011).

Insect - pathogen synergisms are the Foundation of Weed control

Co-existence of the arthropod and fungal biological control agents can be both - beneficial and detrimental to each other

depending on their interactions with the host plant. These prominent interactions might provide models for improving weed biocontrol. There is ample opportunity and need to improve frequency of success and level of impact in weed biocontrol. Enhancement of pathogen infection by insect attacks is a promising idea for biological weed control (Venter *et al.*, 2012). Studies by (Suslow *et al.*, 1982) have shown that multiple-agent projects against weeds have been more successful than single agent release, with the biocontrol agents exerting cumulative impact on the target weed. Leaf scarring by the weevils *Neochetina eichhorniae* and *N. bruchi* enhances infection by the fungus (Fig 3 and 4).

Cercospora piaropi on water hyacinth, *Eichhornia crassipes* at the conclusion of field plot studies (Kluth *et al.*, 2002; Kremer and Kennedy, 1996; Kruess, 2002). Insect/pathogen synergism against leafy spurge as reflected in accelerated mortality of leafy spurge with insect/pathogen combinations in caged studies in the greenhouse. Soil was infested with plant pathogens, leafy spurge was planted in

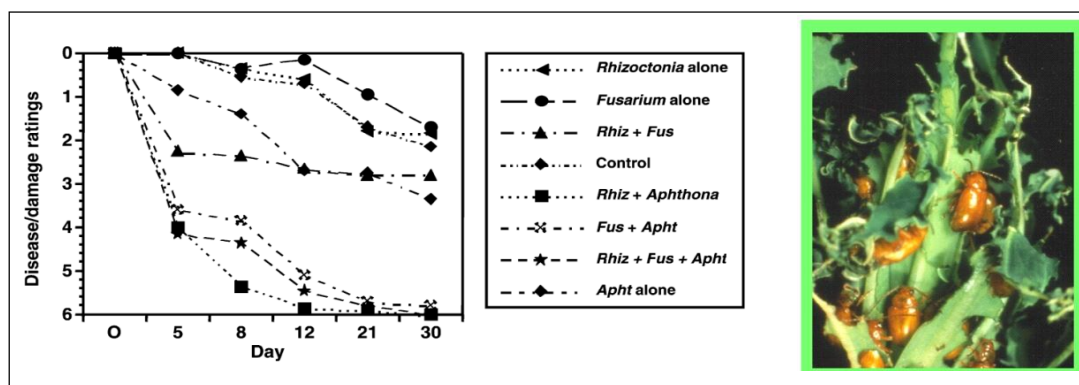


Fig 3: Pathogen/insect synergy against leafy spurge. leafy spurge mortality was accelerated when insect/pathogen combinations were used. A plant pathogen infestation was present in the soil, leafy spurges were planted there, plants were caged and each cage held fifteen insects per plant. Comparable outcomes were observed at multiple high-impact locations where *Aphthona* spp. larvae caused damage to dead and dying leafy spurge where one or more soil-borne pathogens were present. (Caesar, 2000).

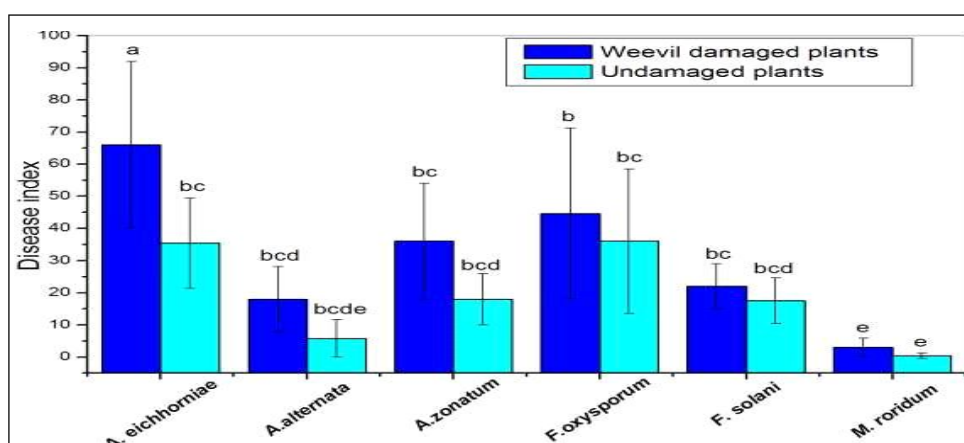


Fig 4: Impact of *Neochetina* feeding on pathogenicity of fungi from South Africa on water hyacinth ♣ Disease index calculated 15 days after application of fungal inoculum ♣ One way ANOVA: Disease index of various fungi on 15th day: $F = 11.01$; $df = 11, 48$; $P = 0.000$.

infested soil plants were caged and 15 insects per plant were placed in cages.

In caged studies in the greenhouse, leafy spurge mortality was accelerated when insect/pathogen combinations were used. A plant pathogen infestation was present in the soil, leafy spurges were planted there plants were caged and each cage held fifteen insects per plant. Comparable outcomes were observed at multiple high-impact locations where *Aphthona* spp. larvae caused damage to dead and dying leafy spurge where one or more soil-borne pathogens were present (Caesar, 2000).

FUTURE THRUST AND OUTLOOK

The future thrust and outlook for R and D should prioritize the evaluation of suitable formulations in bioherbicide agents such as granules, surfactants etc. There is a scope for microbial inoculants for weed control which perform best under specific soil conditions. Thus influencing their use in integrated weed control strategies. Future research should also pave the way to develop biological control agents for highly effective in the suppression of broad spectrum of weeds. Microbial inoculum may be risky and may require science-based evidence to prove the non-target effect of crops and also understand the mechanisms involved in these interactions.

CONCLUSION

It is evident that interactions with biological agents enhance the efficiency and reduce the detrimental effect of weeds, which are mostly suitable and successful for non-cropped situations. This interdisciplinary approach towards finding superior bioagents also covers the longer term evaluation activities that will deliver reliable data and resources needed to culminate in successfully reducing weed populations sufficiently. The challenge is to identify appropriate microbe/bio-agents, which can limit weed growth as well as promote the growth of crop plants, besides having no detrimental effects on crops. Cultural practices to encourage inhibitory bacteria on weed roots (cover crop complementary interaction with DRB) and bioagents limiting competition with crop plants as well as limiting use of synthetic herbicide, so to reduce negative environmental impacts besides herbicide resistance.

Conflict of interest

The authors declare that they have no conflict of interest.

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