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Exploring principles and protocols for biological weed control: A comprehensive review

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Abstract

Biological control involves the targeted application of specific micro-organisms to control infestations of particular weeds, aiming to achieve effective results within the same year of application. The biological approach to weed management is considered more effective and environmentally friendly compared to chemical or mechanical methods, which are often less sustainable. The biological control approach encompasses two main methods: classical and inundative. The classical approach relies on natural enemies that take more than a year to develop effective, weed-suppressive populations, while the inundative method of weed control involves releasing a large number of predators, utilizing bioherbicides, and employing integrated pest management. This approach is less commonly used than the classical biological control method. This chapter delves into the examination of weed ecology, origin, principles, steps, and constraints associated with biological control. It explores why only a limited number of bioherbicides have demonstrated success in field-scale weed control, while others face challenges such as restricted host-range, intricate formulation requirements, and a lack of persistence in both conventional and sustainable agricultural systems.

Keywords: Biological weed, principles and protocols

Introduction

To develop an effective weed control program for a specific area, it is crucial to understand the characteristics and habitat of the weeds present. This includes knowledge of how these weeds respond to environmental changes and react to various herbicides. Before choosing a weed control method, it is essential to gather information on factors such as the quantity of viable seeds, seed dispersal mechanisms, seed dormancy, the longevity of buried seeds, and the ability of weeds to survive adverse conditions. Additionally, consideration should be given to the life span of the weed, soil characteristics (including texture and moisture), and the specific area to be managed. Successful application of certain herbicides depends on soil type; for instance, soil-applied volatile herbicides may be effective in sandy loam soil but not in clayey soil. Similarly, flooding as a weed control method may be successful in heavy soil but not in sandy soil. Weed management encompasses both preventive and curative measures, including eradication strategies, to address the weed problem comprehensively. The goal of a weed management system is to create an environment that is unfavorable for weeds by employing mechanical, biological, cultural, and chemical methods. These methods can be used individually or in combination, forming a holistic approach to weed management. Plants and animals strive to survive individually and ensure the continuation of their species. Human perception categorizes an organism as "beneficial" when it yields food, fiber, or other advantages for humans. Conversely, it is considered a "pest" if it competes with us for resources or affects our comfort. Organisms are typically ignored when they neither benefit nor harm us. When an organism consumes both desirable and pest plants, conflicting interests arise, necessitating risk: benefit analyses to address economic pressures and comply with federal laws protecting native plants and endangered species. The term "control" does not imply the total eradication of a species in a particular area. The required level of control and the tools chosen depend on factors such as the environment, weed infestation, land condition, and land use requirements determined by the land manager. Biological control, also known as Biocontrol, involves the deliberate deployment of living organisms to diminish the population of a pest.

This method encompasses the use of insects, nematodes, mites, plant pathogens, and vertebrates. Multiple Biocontrol agents are often introduced to combat a single weed. Each of these adversaries has some detrimental impact on the weed, ranging from evident effects like defoliation to more subtle consequences, such as minor damage facilitating greater harm inflicted by secondary organisms like pathogens [1].

Understanding the fundamental principles of weed ecology for effective management [2]

The study of ecology, which delves into the interactions between organisms and their environment, is critical for understanding the growth characteristics and adaptations that enable weeds to flourish amidst environmental changes. Human activities, such as modifying crop husbandry practices and maintaining weed-free mono or multi-crop cultures, exert a significant influence on the environment and play a crucial role in shaping this interrelationship. Key aspects of weed ecology that are pertinent to management include how weeds respond to climate, their life cycles, seed biology, and methods of seed dispersal. Weeds possess certain inherent characteristics provided by nature, including:

a) Prolific seed production

- They produce a significantly larger quantity of seeds compared to crops.
- Annual weeds, in particular, are known for generating an extensive number of seeds.
- Seed production ranges from 40 to 196,000 seeds per plant.
- Weed seeds are typically small but contribute significantly to seed reserves.
- Weed seeds exhibit early germination, and their seedlings tend to grow rapidly.

b) Seed Longevity and Viability

- Weed seeds have the remarkable ability to maintain their viability for extended periods, even in unfavorable conditions.
- For instance, lotus seeds retrieved from the depths of the Manchurian lake bed remain viable for up to 1000 years.
- In comparison, wild mustard seeds can stay viable for 30 years, while Chenopodium seeds retain viability for 20 years.

c) Seed Dormancy

- Seed dormancy serves as a highly effective survival mechanism for weeds.
- This state is characterized by the failure of a viable seed to germinate even in conditions conducive to plant growth.
- Most weed seeds display some form of dormancy, such as inherent, innate, or natural dormancy, as well as induced or secondary dormancy. Examples include enforced dormancy by wild mustard, black mustard, and wild oats, each exhibiting distinct types.
- Avena fatua demonstrates the presence of all these various forms of dormancy.

d) Dispersal and Germination of weed seeds

Weed seed dispersal knows no geographical limits. Primary agents responsible for weed dispersal include wind, water, animals, birds, organic manure, agricultural implements, and human activities. The dispersion of mature seeds and live vegetative parts of weeds is a natural process that provides new individuals with non-competitive sites. If there were no natural means of weed dispersal, we would not witness their widespread and robust existence today. Weeds are known as adept travelers, and their effective dispersal involves two key elements: a successful dispersing agent and an adaptation to the new environment. Common weed dispersal agents include wind, water, animals, and humans.

Wind

Weed seeds and fruits dispersed by the wind possess special structures to stay afloat. Examples include *Pappus* (parachute-like modification), *Comose* (covered with hairs), Feathery persistent styles, Balloon (modified papery calyx), and Wings.

Water

Aquatic weeds primarily disperse through water, drifting as whole plants, plant fragments, or seeds with water currents. Terrestrial weed seeds also disperse through irrigation and drainage water.

Animals

Birds and animals play a crucial role in weed dispersal by ingesting weed fruits. Ingested weed seeds pass in viable form with animal excreta, a process known as endozoochory. Farm animals carry weed seeds and fruits on their skin, hair, and hooves, aided by special appendages like hooks, stiff hairs, and sharp spines,

Man

Humans inadvertently disperse numerous weed seeds and fruits through raw agricultural produce. Weeds, maturing simultaneously and at a similar height as crops, are unknowingly harvested alongside crops, contributing to the dispersal of weed seeds. Weeds with this dispersal pattern are termed "Satellite weeds."

Manure and Silage

Viable weed seeds are present in farm animal dung, forming part of the farmyard manure (FYM). Additionally, mature weeds added to compost pits or farm waste act as a source of dispersal.

Dispersal by Machinery

Cultivation machinery like tractors can easily carry weed seeds, rhizomes, and stolons when used in infested fields, subsequently dropping them in other fields and initiating new infestations.

Intercontinental Movement of weeds

Weeds are introduced from one continent to another through various means such as crop seeds, feedstock, packing material, and nursery stock. An example includes *Parthenium hysterophorus*.

Other Distinctive Characteristics

- Synchronization in germination timing between weeds and crop plants is a notable trait.
- They exhibit the ability to germinate under diverse conditions, with a distinct seasonal pattern. The peak

germination consistently occurs in specific seasons year after year.

- Quick responsiveness to available soil moisture and nutrients is a common feature.
- Weeds tend to flower earlier and reach maturity ahead of the crops they infest.
- They show tolerance to shading effects caused by crops during the establishment phase.
- Many weeds utilize the C4 type of photosynthesis, providing an additional advantage during periods of moisture stress.
- They demonstrate relative immunity to soil disturbance practices post-seeding.
- Weeds typically possess extensive root systems, which can grow both deeper into the soil and exhibit creeping tendencies.

Origin of bio control

In the initial attempts to control weeds, there was a heavy reliance on mechanical techniques, crop rotation, and seed cleaning. However, these methods required a considerable amount of labor. A noteworthy shift occurred with the advent of modern organic herbicides, presenting a solution to previously encountered challenges. According to historical accounts, it started from china; the Chinese noticed that an upsurge in ant populations in their citrus groves contributed to a reduction in the numbers of harmful large boring beetles and caterpillars. This application of a natural opponent for pest management marked the beginning of biological control. Biological control research and application hold even greater significance in the present day. The assessment of foreign and indigenous organisms that prey on weeds is underway for their potential use as biological control agents. As a method for managing weeds, biological control provides an eco-friendly approach that complements traditional methods. It addresses the demand for novel weed management strategies, particularly as some weeds have developed resistance to specific herbicides. Notably, biological control agents are designed to target particular weeds, and this technology is deemed safe for both applicators and consumers.

Another illustration of the effectiveness of this efficient method was found in Australia, where 60 million acres of grazing land heavily infested by prickly pear cactus. Vast stretches, covering hundreds of square miles, had become almost impassable for both humans and animals. In response to this challenge, a small moth from Argentina was introduced and released in the country. The larvae of the moth burrowed into the cactus, flourished, and reproduced, resulting in a substantial reduction in the prickly pear population within a decade. Currently, the cactus only occupies 1% of the area it dominated in 1925 [3].

Fig 1: Caterpillars of the moth *Cactoblastis cactorum* bore into the pads of prickly pear. This dameges the cactus and introduces a bacterium that causes the plant to die (Photo provided by E. S. Delfosse, USDA / ARS)

Principles and Protocols of Biological Weed Control

The foundational concept guiding the biological approach to weed control is rooted in research indicating that exotic plants turn invasive due to their evasion of insect herbivores and other natural adversaries that typically restrict their growth and spread in their native habitats. Although various factors may contribute to the inclination of certain plant species to become invasive, biological control entails the utilization of specific natural enemies capable of inhibiting the growth and reproduction of the target organism, thereby imposing limitations on them. The utilization of biological weed control does not negate the necessity for chemical herbicides. It is essential to integrate both these methods with cultural practices, including tillage and crop rotation, in the ongoing effort to combat weeds. Through the implementation of Integrated Weed Management, the emergence of weeds that develop resistance to biological or chemical agents can be effectively delayed [4].

There are four approaches to biological weed control

a) **Classical method:** It involves introducing a non-native organism, typically an insect, into areas afflicted with the targeted weed. The Biocontrol organism feeds on the weed, gradually decreasing the weed population over time.

b) **Inundative method:** It entails cultivating an organism in a controlled environment and then releasing it in large numbers to manage native or invasive weeds.

c) **Conservation approach:** It involves manipulating a cropping system to enhance the populations of natural organisms that suppress weeds.

d) Grazing technique employs large herbivores like cattle or sheep to diminish weed populations.

1. Classical method

The initial step in implementing a biological weed control program involves assessing the suitability of the targeted weed. Not all weeds are conducive to biological control. Weeds which closely related to economic crops are not well-suited for this method. The closer the relationship, lower the likelihood that a biotic agent could effectively distinguish between the weed and the crop. Classical Biocontrol involves the identification and release of a natural predator to manage the spread of invasive weed species. Various biological control tools for weeds include insects, mites, nematodes, pathogens, grazing animals and insects which have a direct effect on weeds by diminishing their growth and competitiveness. Furthermore, specific organisms employed in biological control focus on weed seeds, preventing their incorporation into the soil seed bank. The primary objective of classical biological control is to lower weed density to a point below the economic threshold, specifically below the level where economic damage occurs, careful consideration and thorough risk assessments are essential to avoid unintended consequences and potential harm to non-target species. Additionally, regulatory approval is often required before introducing natural enemies into a new environment. Thorough research is conducted to identify appropriate natural enemies for the targeted pest. These natural enemies should be specific to the pest species and pose minimal risk to non-target organisms. The process of identifying and introducing biological control agents involves several steps:

- Identification of target weeds.
- Identification of control agents and assessment of their level of specialization.
- Controlled release of the agents.
- Full release and identification of optimal release sites.
- Monitoring release sites.
- Redistribution in the case of classical methods.
- Maintenance of control agent populations.

Once identified, the natural enemies are brought in or collected from their native habitat and released into the affected area. This step requires careful consideration of factors such as climate, ecosystem dynamics, and the life cycle of both the pest and the natural enemy. Followed by release, the natural enemies are allowed to establish themselves and build populations. Several considerations play a crucial role in the selection of sites for the release and establishment of insect biocontrol agents. The chosen site must be devoid of chemical treatments, including insecticides and herbicides, or, at the very least, must have no future plans for the use of these chemicals that could disrupt the life cycle of the biocontrol agent. Additionally, the field should be free from grazing animals during the initial establishment of the biocontrol agent, although wildlife grazing is unlikely to pose a threat to the developing colony. Installing fencing around the release site may be the most effective measure to prevent disturbance and provide the agents with the necessary time to establish themselves. Continuous monitoring is essential to assess their effectiveness, evaluate their impact on non-target species, and make any necessary adjustments over time; the natural enemies may adapt to the new environment and develop characteristics that enhance their efficacy in controlling the target pest. Monitoring is contingent on how the agent utilizes its host, the anticipated density of the agent during sampling, the life cycles of both the agent and host, the required precision of the data sought, and the level of effort, workforce, and financial resources available for the sampling process. Successful biological control programs can lead to sustainable, long-term pest management, reducing reliance on chemical pesticides and promoting environmentally friendly agricultural or ecological practices [5].

2. Inundative method

Method involves the mass release of a large number of biological control agents (such as predators, parasites, or pathogens) into an area infested with pests. The aim is to quickly overwhelm the pest population and achieve effective control. This approach is often employed in situations where the pest population is at a level that necessitates rapid and substantial intervention. The released biological control agents are carefully selected to be specific to the target pest, minimizing the impact on non-target organisms. It is often considered a short-term strategy, as the released agents may not establish long-term populations. Multiple releases may be needed to maintain control. This method is suitable for situations where there is an urgent need to address a pest outbreak, such as in agriculture or forestry. The success of inundative biocontrol depends on various factors, including the timing of releases, environmental conditions, and the compatibility of the biological control agents with the target pest and the ecosystem. Additionally, it may be necessary to repeat releases to sustain effective control over time [5].

3. Conservation method

Conservation biocontrol relies on understanding the biology and habitat suitability of the beneficial insects or rodents that feed on weeds or weed seeds. In order for a beneficial insect or rodent to contribute to weed control, the habitat (i.e. field) must meet its needs (i.e. for adequate food and shelter). With this knowledge, management practices are adjusted so that these organisms are promoted or encouraged. Establishing a winter cover crop using no-till is a conservation biocontrol practice because it protects invertebrates in the cropping system, such as ground beetles, which consume weed seeds on the soil surface. Establishing windrows that provide habitat for rodents such as field mice is another conservation biocontrol practice. Both insects and rodents readily feed on weed seeds, potentially reducing the number of weeds that emerge the next year. The quantity of seeds consumed is contingent on factors such as the populations of predators, the availability of weed seeds, and the practices employed in field management. Decreasing soil disturbance and providing ground cover or refuge from predators is one of the key ways to conserve these naturally occurring biocontrol organisms. This approach creates favorable habitats for the insects and rodents already present in an area. These organisms are then active when weeds are vulnerable. Organisms that feed on weed seeds (weed seed predators) are active when weed seeds are maturing and after they are dispersed Sole use of this method of biological control will not completely suppress weeds and limit crop yield loss. However, the combination of conservation biocontrol with other cultural, mechanical or chemical management tactics could have a greater positive impact. Conservation methods for weed control aim to minimize the reliance on synthetic herbicides, reduce environmental impact, and promote sustainable agricultural and ecological practices. Implementing a combination of these methods, tailored to specific conditions and weed species, is often a key to successful weed management. Many organisms serve as predators of weed seeds, with rodents, ants, crickets, and ground beetles being among the most prevalent and effective. For example mice exhibit opportunistic feeding behavior, targeting readily available, high-density food sources. Consequently, seeds constitute their main dietary component. Within a 12-hour timeframe, mice have the capacity to ingest 90 to 100% of a given area's weed seeds. These rodents employ their olfactory senses to locate seeds and can adeptly uncover seeds buried beneath the soil surface. However, mice can pose challenges in certain agricultural systems by consuming crop seeds. Additionally, they may cause disturbances to irrigation equipment, plastic mulch, and other agricultural tools [5].

4. Grazing method

Grazing is an ecological practice employed in weed control, utilizing the feeding activities of animals to manage weed populations in various environments. This method is particularly relevant in agricultural, rangeland, and natural ecosystems. Grazing can have varying effects on weed abundance at a specific location. On its own, grazing is unlikely to completely eradicate invasive plants. However, when grazing interventions are combined with other control methods, such as herbicides or biocontrol, they can significantly reduce severe infestations and potentially eliminate smaller ones. Grazing animals can be especially beneficial in areas where herbicides cannot be applied, such as that near water, or in situations where herbicide costs are prohibitively high, such as with extensive infestations. Additionally, animals can play a role in restoration programs by cultivating the soil and facilitating the incorporation of seeds from desirable native plants. To optimize the weed control benefits of grazing animals, it may be necessary to use fencing to adjust grazing pressure. This involves increasing the number of animals and the duration of grazing at strategic times during the growing season to prevent selective grazing, where animals choose certain plants over others. By doing so, they are compelled to consume more undesirable species. The effectiveness of this weed control method lies in concentrating livestock on weed infestations during crucial stages of weed growth while keeping them away from pastures or weeds during other times. The window for using grazing animals for weed control is limited to periods between crops or shortly after crop establishment, when grazing is tolerable, and the crop can recover rapidly. Monitoring grazing closely is essential, and animals should be promptly removed once adequate control is established or before native species are adversely affected. As a result, land managers need to be adaptable and have the ability to regulate herd movements. Failure to maintain control may lead to overgrazing of desirable species, potentially promoting weed infestations or the establishment of new weed species [5].

Biological control using fungi

Fungi are simple microorganisms lacking chlorophyll. They obtain nutrients from living plants and animals or decaying organic matter, and enter plants through wounds, natural openings, or by penetrating directly through the plant tissue. Fungi cause local or general disintegration of plant cells or tissue, stunting, or abnormal vegetative growth. They may affect plant growth by removing or blocking nutrients to the plant or by producing toxins that can affect the structural and metabolic activity of the plant. Numerous fungi have been officially recognized and approved as bioherbicides intended for application in agriculture or the management of ecosystems. Several examples include species within the genus *Colletotrichum*, Including *C. truncatum*, which has been investigated to control hemp sesbania (*Sesbania exaltata*, *C. orbiculare*, which was investigated for its potential to control spiny cocklebur (*Xanthium spinosum*). Others include genomes of *C. gloeosporioides* and *C. orbiculare*, associated with pathogenesis, including plant cell wall degrading enzymes. In addition to this three species belonging to the *Phoma* genus have garnered interest as potential candidates for biological weed control e.g. *P. herbarum*, initially discovered as a fungal pathogen in dandelion leaf lesions in Southern Ontario, has undergone research for its effectiveness in managing dandelions within turf environments [6, 7].

Similarly, *P. macrostoma* has been studied for comparable purposes, demonstrating a specific inhibitory effect on the growth of dicots [8]. Combining fungal bio herbicides, also known as mycoherbicides, with pesticides can either amplify or diminish the severity of the diseases they induce. Some adjuvants may significantly heighten disease severity by facilitating pathogen penetration into plants, a process that would otherwise be challenging. Certain growth regulators have also demonstrated the ability to improve the efficacy of bioherbicides. In a few cases, it has been observed that sunscreens contribute to extending the shelf-life of bioherbicides, likely by shielding the active agents from harmful ultraviolet radiation. Further incorporation of rain fastness agents could enhance the effectiveness of certain bio herbicides [8].

Management using bacteria

Bacteria are microscopic single-celled organisms that are typically observable only under high-powered microscopes. They undergo rapid reproduction through uncomplicated cell division and access plant tissue through wounds or natural openings. Once inside, they proliferate swiftly, leading to consequences such as cell death, abnormal plant growth, or tissue breakdown. Some bacteria also generate toxins. The effective spread of bacteria occurs through cultivation, rain, flowing water, wind, dust, and the transportation of infected plant material. Several bacteria have been explored as potential agents for biological weed control, with notable attention given to *Pseudomonas fluorescens* and *Xanthomonas campestris*. Biological weed control using bacteria is considered advantageous compared to fungi, thanks to factors such as the quicker growth of bioherbicide agents, relatively straightforward propagation requirements, and high suitability for genetic modification through mutagenesis or gene transfer. *Pseudomonas fluorescens*, in particular, has been extensively studied as a biological weed control agent. Within this species, various strains exhibit either beneficial or inhibitory effects on. Among these strains, three have been thoroughly investigated for their suppressive effects, demonstrating the ability to inhibit plant growth and germination through the production of extracellular metabolites. Another bacterial species that has been extensively studied as a potential agent for biological weed control is *Xanthomonas campestris*. Particularly noteworthy within this species is the strain *X. campestris pv. poae* (JT-P482), which was officially registered in Japan in 1997 for the control of annual bluegrass [9].

Incorporating Biocontrol into Comprehensive Control Approaches

While biocontrol is commonly viewed as a substitute for various methods, especially herbicides, it can effectively be employed in conjunction with them. Incorporating biocontrol with additional control methods is crucial because a weed infestation can proliferate in density and area more rapidly than the newly introduced biocontrol agents can establish populations. As a result, the use of supplementary control methods is necessary alongside the introduction of biocontrol agents. These combinations may either impede or augment each other. For instance, prescribed fires have the potential to significantly decrease populations of biocontrol agents if ignited when the agents are vulnerable and unable to escape. However, careful adjustments in the timing, frequency, and spatial distribution of the burns can mitigate any interference or harm to the agents. In fact, these adjustments may even enhance the overall impact of the biocontrol measures. The application of herbicides can either hinder or boost the effectiveness of biocontrol. The timing of herbicide applications plays a crucial role in the dynamic between biocontrol agents and host plants. It is essential to apply herbicides when their impact on the host plants will not disrupt the life cycle of the biological control agents [10].

Constraints in biological weed control

Despite scientific evidence supporting the effectiveness of biologically based herbicides, companies show limited interest in developing these products, and obtaining patents for their use is often challenging. Introducing insects to manage exotic weeds is a time-consuming process, with a single agent rarely fully suppressing the targeted weed. Some researchers have reported instances where agents introduced for exotic weed control attacked non-target native plants, raising concerns among biological control workers, weed scientists, and governments. In agro-systems, a complex of multiple weed populations exists, and biological strategies may only control one weed. The use of multiple agents extends the time needed for research and development. While numerous bio-herbicides display potential, only a few have achieved lasting commercial success, partly due to challenges in maintaining consistent effectiveness under real-world field conditions. Recent global research and weed management practices challenge the misconception that the biological approach to weed control is inherently slowed, unpredictable, expensive, and largely ineffective. Despite higher initial expenses, the biological method is comparatively more economical than alternative strategies. However, factors like the extended time for establishment (typically 20 years or more), inadequate records of pre-biological control weed infestations, and discouraging accounts of poorly executed bio-control initiatives hinder widespread adoption. Opposition to the biological approach to weed control has further contributed to a slow adoption rate, with some researchers and weed control scientists expressing difficulty in estimating the cost or feasibility of biocontrol.

Conclusion

Despite the high efficiency of chemical herbicides in weed control, their detrimental effects on the environment have led to a need for a shift towards a management model that is environmentally and economically cooperative. This shift has given rise to biological control. A comprehensive understanding of weed competition and weed ecology is crucial for effective implementation and optimization of the efficiency of biocontrol agents. Biological control offers cost-effectiveness, environmental safety, self-perpetuation, and compatibility with integrated weed management programs. However, it is not always immediate or universally adequate, suitable for only certain weeds, and the failure rate can be high. Two approaches are widely used in the model. The classical method of biological weed control, involving the introduction and release of agents such as exotic insects, mites, or pathogens, has been the most popular and widely adopted approach, providing permanent control. The inundative method of biological weed control involves the release of predators, the use of bioherbicides, and other integrated pest management techniques, which are not as widely utilized as the classical method.

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