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Combinations of Microbial and Insect Biocontrol Agents for Management of Weed Seeds

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Abstract

Important roles for biotic agents in integrated weed management include preventing seed production and weed emergence from the seed bank. Seed-attacking microorganisms have been described for a limited number of economically important weeds and serve as examples illustrating the potential for reducing weed seed production. Innundative releases of seed-feeding insects have also successfully reduced viable seeds produced by specific weeds and subsequently reduced the viable weed seed content in soils. There are some indications that seed attacked by either microorganisms or insects carry over detrimental effects in soil and result in reduced weed seedling emergence in the following growing seasons. Combinations of different biotic agents could enhance efficacy of weed management over that observed with either agent alone. For example, a selective seed-feeding insect, *Niesthrea louisianica*, combined with seed-attacking fungi (*Fusarium* spp.) significantly decreased velvetleaf (*Abutilon theophrasti*) seed viability and seedling emergence compared to either the insect or fungi alone. Insect-attacked, fungal-infected velvetleaf seeds survived at very low rates in soil (ca. 2% viable seeds at 24 months.) and in plots where the seedbank was not replenished, no viable seeds were retrieved from soil after 24 months. Integration of compatible biotic agents can be an effective method for reducing seed viability before and after entry into the seed bank. Prevention of seed production is considered the foundation for successful weed management — effective insect + microorganism combinations are key in building this foundation in biologically-based weed management.

Keywords: biological weed management, seed fungi, seed-attacking insects, integrated biological control, seedbanks

Selection of “successful agents” is a central issue to biological control of weeds. Classical approaches for selecting successful biological control agents have focused on “points of attack” on the target weed where damage will likely result in reduction in plant vigor and reproduction or even plant death (Rees *et al.* 1995). Thus, specific insect attributes have been developed to quantify criteria to aid in selection of potential biological control agents (Goeden 1983, Harris 1973). A characteristic that is scored high in this system is the ability to attack seeds to the extent of preventing seed production. A cursory examination of the literature reveals that a majority of insects selected or under study for potential biological control of weeds possess the ability to damage seed production in one of a variety of manners. Several representative biological control examples are listed in Table 1 to illustrate the range of weeds that are vulnerable to seed attack by specific insects. An additional criterion that merits a high score is the ability of insect damage to promote invasion by disease-causing organisms. Interestingly, in the review of literature, very little information regarding observations of microbial associations with seed damage by insects was available. Perhaps this lack of information will be alleviated in the future as biological control scientists adopt a uniform documentation system for recording characteristics of biological control agents (Coulson 1992). It is also possible that the lack of reports on insect-microorganism interactions on weed targets are due to strict quarantine procedures that eliminates any associated microorganism to be released with the insect agent.

Prevention of seed germination and seedling emergence is fundamental to maximally effective long-term weed management (Aldrich and Kremer 1997). Thus, biological control can play a significant role in reducing weed infestations by depleting seeds and

Table 1.
Selected insect biological control agents that attack seeds of weed hosts[a].

Weed		Insect	
Gorse	*Ulex europaeus* L.	Gorse seed weevil	*Apion ulicis*
Knapweeds	*Centaurea* spp.	Lesser knapweed flower weevil	*Larinus minutus*
Yellow starthistle	*Centaurea solstitialis* L.	Yellow starthistle hairy weevil	*Eustenopus villosus*
Puncturevine	*Tribulus terrestris* L.	Puncturevine seed weevil	*Microlarinus lareynii*
Purple loosestrife	*Lythrum salicaria* L.	Blunt loosestrife seed weevil	*Nanophyses brevis*
Scotch broom	*Cytisus scoparius* (L.) Link.	Scotch broom seed weevil	*Apion fuscirostre*
Tansy ragwort	*Senecio jacobaea* L.	Ragwort seed fly	*Botanophila seneciella*
Musk thistle	*Carduus nutans* L.	Thistle head weevil	*Rhinocyllus conicus*
Toadflaxes	*Linaria* spp.	Toadflax capsule weevil	*Gymnetron antirrhini*
Parkinsonia	*Parkinsonia aculeata* L.	Parkinsonia seed bruchid	*Penthobruchis germaini*
Mesquite	*Prosopis* spp.	Mesquite seed weevil	*Algarobius prosopis*
Silky hakea	*Hakea sericea* Schrad.	Hakea seed weevil	*Erytenna consputa*
Lantana	*Lantana* spp.	Lantana seed fly	*Ophiomyia lantanae*
Broomrape	*Orobanche cumana* Walt.	Broomrape seed fly	*Phytomyza orobanchia*
Velveleaf	*Abutilon theophrasti* Medik.	Scentless plant bug	*Niesthrea louisianica*
Pigweeds	*Amaranthus* spp.	Seed moth	*Coleophora lineapuvella*

[a] Examples compiled from Julien and Griffiths (1998) and Rees *et al.* (1995).

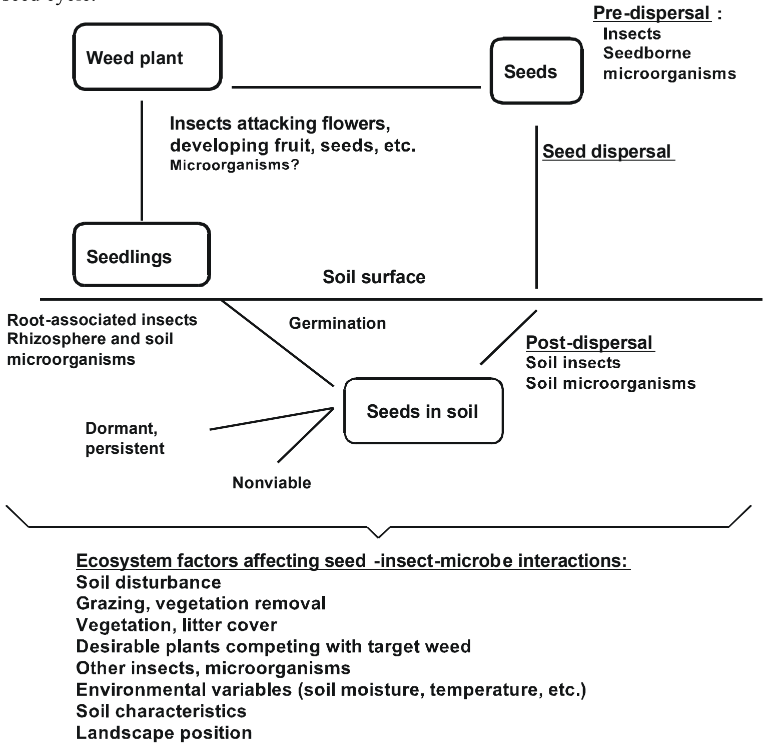
seedlings before they become competitive with desirable plants. Releases of seed-feeding insects have successfully reduced viable seeds produced by specific weeds (Julien and Griffiths 1998) and in some cases the viable weed seed content in soil (seedbank) was also subsequently reduced (McEvoy *et al.* 1990). Unfortunately, in most cases, opportunities to investigate and document the impacts of associated microbial activity directly with the insect agent or indirectly with the damage inflicted by the insect were missed.

This review explores the potential of integrating insect biological control agents with microorganisms for attacking weed seeds as a valid method of enhancing or augmenting management of weeds in a biologically based system. The relationship between insects and microorganisms in damaging weed seeds has not been thoroughly explored. An understanding of these relationships will be described from which potential biological weed management strategies can be developed.

Role of insects in reducing weed seed numbers and viability

Biological control based on reduction of reproductive capacity of weeds by insect agents involves damage inflicted at the flowering to immature seed phase, at pre-dispersal prior to release of mature seed from the plant, at post-dispersal when seeds are on the soil surface and within the soil, and at seed germination and emergence (Fig. 1). This review will focus on pre- and post-dispersal stages of attack; for information on attack at

Fig. 1. Involvement of seed-attacking insects, microorganisms, and ecosystem factors with the weed seed cycle.



flowering to immature seed stage, the reader is referred to Julien and Griffiths (1998) for descriptions of the various insects and target weeds involved. Examples illustrated in Table 1 are typical of selections of insects for pre-dispersal seed attack. In each case incidental observations of possible involvement of microorganisms in the seed damage incurred were not reported. It is also notable that occasionally some widely established biological control insects may not be as effective in causing damage to host weeds as originally thought. For example, the seed fly *Ophiomyia lantanae* that feeds on the fruit of *Lantana* spp., caused very little damage to viability of embryos within the fruit (Broughton 1999). This is a situation where selected microorganisms could augment effect of the insect. Also, McEvoy *et al.* (1990) documented that combined effects of two different insects on depression of tansy ragwort densities and seed production was greater than either agent acting alone. However, the growth suppression was offset by soil disturbance or grazing resulting in abundant production of viable seeds for restoration of a seedbank under depletion due to germination and mortality. Seedborne and soil microorganisms could contribute to the seed dynamics in this situation but the impact of microorganisms was not reported. Most recently larvae of a "micro-moth" has been discovered that feeds in the seeds of *Amaranthus* spp. while on the plant (Griffiths and Swanton 1999). The significance of pre-dispersal feeding on this weed is that not only are viable seeds potentially reduced from adding to the seedbank but also seeds of this weed species that is prone to develop resistance to herbicides can be eliminated thereby reducing future infestations of herbicide-resistant biotypes.

Due to recent interest in establishing biologically-based management addressing all phases of weed development, considerable effort has been expended to understand and exploit post-dispersal weed seed dynamics. Predation of weed seeds dispersed to the soil surface is considered a potential approach to reduce viable seed numbers entering the seedbank. Up to 57% of velvetleaf seeds were removed from the soil surface by predators, including carabid beetles (*Amara* spp.) which also damaged intact, dormant seeds suggesting potential decreases in the persistent component of the seedbank (Cardina *et al.* 1996). Carabid seed predators were also shown to be nonspecific in feeding on seeds of various weed species and may be considered a major factor in natural biological control affecting weed population dynamics (Cromar *et al.* 1999). Other weeds where post-dispersal predation is reported to be significant in reducing seedbank inputs and subsequent reduced seedling emergence include *Chondrilla juncea* attacked by seed-harvesting ants (Panetta 1988) and johnsongrass (Van Esso and Ghersa 1989).

Microorganisms and effects on weed seeds

Characterization of seedborne microorganisms of various weeds has been presented previously (Kremer 1993). It was noted that the microorganisms associated with weed seeds prior to and upon entry into soil likely contribute to depletion of at least a portion of seedbank with the possibility of increasing seed attack by these microorganisms once a better understanding of the deterioration mechanisms of weed seeds in soil is obtained. It should be noted that some fungal pathogens are under development as potential mycoherbicides for pre-dispersal attack of weed seeds. The loose smut fungus *Sphacelotheca holci* readily infects seed heads of johnsongrass (*Sorghum halipense* [L.] Pers.) to reduce seed viability and seed set, biomass accumulation and rhizome extension, and ultimately reduce the seedbank (Massion and Lindow 1986).Competitiion of johnsongrass with crops enhanced infection and reduction in plant vigor. An extension of this system would be to determine whether seed predators might carry infected (smutted) seeds into soil, promote infection, and ultimately reduce seed numbers in the seedbank. Another loose smut fungus, *Ustilago syntherismae*, infects crabgrass (*Digitaria* spp.) systematically to reduce plant vigor and reduce seed production with up to 88% plants infected with smut (Johnson and Badouin 1997). Involvement of appropriate insect feeders might improve the level of seed mortality.

When considering a weed biological control strategy based on seed attack, one must remember that unlike pathogens developed for inundative biological control as bioherbicdes that are selected for quick, complete and easy weed kill during a crop growing season similar to the effect of herbicides (Charudattan 1990), pathogens selected for seed attack should be viewed as a long-term measure for weed management. The efficacy of microbial seed attack can be improved by combining with selective seed-attacking insects. For example,a strategy for prevention of weed seed production and subsequent reduction of the seed bank could be attained using a bioherbicide consisting of seed pathogens applied to grass weeds occurring in a crop (Medd and Campbell 1996). It is very likely that suitable insect seed-feeders could be identified for the target grass weeds to augment seed infection by the applied pathogens. However, the impact of the bioherbicide (and potential associated insects) would not be evident for one to two years until noticeable decreases in weed seedling densities occur due to the reduced seed bank size.

Integration of biological control agents

Damage to target weeds could be enhanced or augmented by integrating biological methods such as use of microbial biocontrol agents with seed-attacking insects under the concept of "biological weed management" (Cardina 1995). It is obvious from past performances of biological control that single-tactic approaches often have not been effective in long-term management and has sometimes resulted in weed shifts, replacement with more competitive species, and development of resistant populations. Therefore, integration of multiple tactics, including a diversity of biological agents and approaches, favors the effectiveness and stability needed for long-term weed management. The aim of integrated approach is to manage weed population at an acceptable level by reducing: a) number of propagules; b) number of emerging seedlings; and c) survival and competitiveness of emerged weeds.

Although the idea of combining microbial and insect approaches for improved efficacy of biological control has been largely anecdotal over the years, it was Spencer and Sankaran (1985), while documenting potential insect and pathogen biological control agents discovered for velvetleaf in India, who suggested that a team approach combining the disciplines of entomology and plant pathology would be effective in evaluation of the potential for finding biocontrol agents. Indeed it was the integration of the scentless plant bug (*Niesthrea louisianica*) and pathogenic fungi for attack of velvetleaf seed that demonstrated the successful use of an approach integrating both insects and microorganisms (Kremer and Spencer 1989a, b). The selective seed-feeding scentless plant bug attacks immature velvetleaf seeds and reduced seed viability to 16% compared to 96% for insect-free seeds. The insect also enhanced microbial infection in seeds up to 98% compared to 8% infection for non-attacked seeds. When seeds were buried in soil, seed viability continued to decrease to ca. 2% after 24 mo. These results prompted a subsequent study examining the deliberate integrated use of the two organisms for effects on seed viability. Developing seed capsules on velvetleaf in field were sprayed with suspensions of selected seed fungi (*Fusarium* spp.). After fungal application, insects were released onto half the plants contained in cages and allowed to feed throughout the growing season. *Fusarium* spp. alone had little impact on viability. Insects attacking seeds without *Fusarium* spp. reduced viability to 25% and increased infection with naturally occurring fungi to 58%. Combined fungal application plus insect feeding further reduced seed viability to <2%, and infection with the selected fungus increased to 98%. When seeds harvested from the treatments were planted in soil, seedling emergence was 66, 8 and 5% for control, insect-attacked, and insect + fungi treated seeds, respectively (Kremer 1995) thus demonstrating potential reduction of viable seeds in the seedbank. These results demonstrated that integration of compatible biological agents is an effective method for reducing seed viability prior to entry into the seedbank.

Few other reports of integrating microbial and insect agents also demonstrate enhancement or augmentation of detrimental effects on seed viability. Chrysomella beetles (*Chrysolina hyperici* Forest.) can augment biological control of St. Johnswort (*Hypericum perforatum* L.) seedlings by transmitting the fungal pathogen *Colletotrichum gloeosporioides* f. sp. *hypericum* (Penz.) Penz. and Sacc. during foraging and feeding (Morrison *et al.* 1998). The fungus can reside in infected seeds and vegetative residues thereby providing a reservoir of inocula for future infestations. The foliar rust fungus, *Puccinia carduorum*, screened for efficacy on musk thistle significantly reduced viable seed production when in combination with seed-feeding insects on the plants (Baudoin *et al.* 1993). As would be expected, the fungus alone has little impact on seed production. Research to determine why reduction in seed production is enhanced by combinations of seed-feeding insects and a foliar pathogen is underway. This study indicates that potential effective combinations are not limited to specialized seedborne pathogens. The potential of soil microorganisms in further reducing viability of seeds attacked by insects prior to dispersal was illustrated in a study of the fate in soil of puncturvine (*Tribulus terrestris* L.) seeds that had been attacked by a seed-feeding weevil (Goeden and Ricker 1973). Infection by soil microorganisms killed the majority of weevil-damaged seeds, even though the loss of intact , undamaged seeds was low in all soils and depths studied.

Environmental and management factors

In agroecosystems several cultural practices can be used to augment the effects of integrated biological control approaches. Practices that modify weed density and seed production can favor efficacy of seed attack. For example, crop rotation schemes combined with reduced tillage resulted in lowest seed production of grass and broadleaf weeds in a corn- soybean-wheat-alfalfa rotation compared to continuous corn or corn-soybean (Kegode 1999). Also, crop rotations, cover cropping and field border vegetation promote ground beetle populations as components of biological weed management. This was reflected in post-dispersal seed predation by ground beetles (*Harpalus rufipes* DeGeer) in potato (*Solanum tuberosum* L.) production fields which reduced seedling emergence of several annual weeds including redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.) by not only direct feeding but also by caching seeds into the soil by burrowing larvae at depths unfavorable for germination or vigorous seedling development (Hartke 1998).

Density of target weeds in agroecosystems or natural ecosystems affects seed production as well as activity of biocontrol agents. For example, the fungal agent, *Colletotrichum coccodes*, had little influence on velvetleaf seed yield in pure stands whereas in soybean field, seed production was reduced 52 to 62% (Ditommaso *et al.* 1996). The increased seed mortality was likely due to competition of soybean causing stress on velvetleaf growth thereby lowering overall plant vigor. Similarly, effectiveness of the scentless plant bug and seed fungi is reduced in dense stands of velvetleaf where both agents are unable to consistently attack all seed capsules developing continuously over time (Kremer 1992). Management of ground cover (vegetative residues) is also critical in providing suitable habitats for seed predators in either agricultural or natural ecosystems (Reader 1991). It should be noted that management factors that affect weed seed production and effects of biological agents affect soil microorganisms, which subsequently affect fates of seeds dispersed to the soil (Turco *et al.* 1990).

Summary

Knowledge of the impacts of the above factors on integrated biological control of seed production can lead to approaches to maintain natural biological control without necessity of releasing agents into the environment. This requires an understanding of the ecosystem and dynamics of beneficial organisms in it. It may be possible that management systems might be developed that encourage natural seed-attacking organisms at all levels of the seed cycle (immature development, pre-dispersal, post-dispersal, seedbank) that are based on balancing the ecosystem factors that are imposed on the weed seed dynamic model (Figure 1). Exploitation of these ecological aspects of weed seed-microorganism-insect relationships will lead to progress toward management systems for both natural and agricultural ecosystems.

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