

The Importance of Intertrophic Interactions in Biological Weed Control

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ABSTRACT

The earliest research leading to successful weed biocontrol included observations and some analysis that the strict “gate-keeping” by peer reviewers, editors and publishers does not often allow today. Within these pioneering studies was a valid picture of the biology of weed biocontrol that is applicable today. Two major studies pointed to successful weed biocontrol of perennials as an outcome of intertrophic interactions. Later work indicated that there was a consistent association of certain fungal species with insect damage. In recent years, ecological studies have provided evidence of the effect of the soil microbiota in combination with root herbivory on plant community structure and on invasiveness. This accretion of evidence and the authors own findings have led to the conclusion that in selecting agents for biocontrol of exotic perennial invasive plants, the capacity of the agent to synergistically interact with other agents should be included in the criteria. If the hypothesis that insect/pathogen interactions underlie successful biocontrol of herbaceous perennial invasive plant species, then efforts to restore native plants would be affected by the biotic legacy of the interactions. Findings from a post-biocontrol native plant restoration have provided such evidence. The existence of insect/pathogen interactions provides a unique position for plant pathogens as being an important factor prior to, during and after biocontrol.

Keywords: biotic legacy, follow-on effects, insect/pathogen, invasive weeds, plant-soil feedback, root herbivory, restoration, soilborne pathogens, synergism

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INTRODUCTION

Insect/plant pathogen interactions for the biological control of exotic invasive weeds present interesting benefits to the practice of classical weed biocontrol in general. Such interactions may be key to preventing resurgence of weed populations after the initial effects of weed biocontrol from release and establishment of insects resulting in the initial take-down of weed infestations has occurred. Close study of such herbivore/pathogen interactions may also yield information that can be applied in programs to supplement sites where insects established but had not impacted weed density. Impact as indicated by the formation of a “halo” of lower weed density or achievement of more than a 10% decrease in stand density was found in one post-hoc study to be between 24-60% of sites where *Aphthona* spp. were released against leafy spurge (Kalischuk *et al.* 2004). In another study, sites that were at least moderately impacted as perceived by land managers were ca 60% of all release sites of *Aphthona* spp. (Hodur *et al.* 2006). That is, there remained as of the dates of these studies a large percentage of infestations that are unimpacted by insect-centric biocontrol, between 40-76%. In general, it has been concluded in still other studies that the results of insect releases on

spurge density are highly variable (Butler *et al.* 2006; Larson *et al.* 2008). However, the capacity to achieve even this range of success may be dependent upon the ability of *Aphthona* spp. and other root herbivorous insects specific to leafy spurge to provide conditions for insect synergisms to develop (Caesar 2003). Future biological control efforts against perennial invasive and exotic weeds could benefit by use of research findings that support recognition of an essential role of soilborne plant pathogens and deleterious bacteria in synergisms with insects.

THE ORIGINS OF INSECT/PATHOGEN INTERACTIONS AS A MEANS FOR SUCCESSFUL INVASIVE WEED BIOCONTROL

This author’s interest in insect/pathogen interactions developed from the first biocontrol site observed, in 1991. After the snow had finally melted in mid-May of that year, a colleague visiting a stand of the exotic herbaceous perennial leafy spurge in Bozeman, Montana, where the flea beetle *Aphthona flava* had been released 4 years prior, found that the stand density was dramatically decreased. The stand showed the dramatic effects of having of being “cleared” of spurge in the center of the population on a south-facing

hillside. That the effects occurred in the central portion of the stand was another story, which will be discussed later. The cleared portion was more or less circular, which was later often referred to as the “bomb blast” when discussing the first such signs of effective biocontrol of leafy spurge. In tracing the periphery of the cleared area, there were shoots of spurge that were wilted or dead. The roots of such plants were damaged by larvae of *A. flava*. The samples collected nearly all yielded *Rhizoctonia* spp., *Fusarium* and *Pythium* spp. when isolations from root tissue were done in the laboratory. In subsequent work, similar results were obtained at about 40 such sites where spurge was decreasing in density following release of *A. flava* or other *Aphthona* spp. In the native range of *Euphorbia esula/virgata*, where populations are generally either small and limited in area or sparse and scattered, isolations from roots with insect damage caused by *Aphthona*, *Chamaesepelia* or *Oberea* spp., yielded the same complex of soilborne pathogens. Thus, insect feeding provided avenues for infection by soilborne plant pathogens, which had accrued in the soil during the several years the spurge plants had infested the site. Findings such as this could be considered as unsurprising by plant pathologists since insect/pathogen or nematode pathogen synergisms are an integral part of their undergraduate and graduate studies. The nature of this connection between insect damage and root disease was further elucidated by studies of the comparative virulence of isolates of *Rhizoctonia* and *Fusarium* spp. from leafy spurge and spotted knapweed in their invaded and native ranges (Caesar 1994a, 1996; Caesar *et al.* 1998, 1999, 2002) and surveys in the native range of white top (*Lepidium draba*) (Caesar *et al.* 2010) (Caesar and Caesar-TonThat 2008). Insect/pathogen associations which are similar to the leafy spurge/insect/plant pathogen system include *Alternaria* blight of rubber trees, blue stain on conifers, Dutch elm disease, lint rot of cotton, internal boll disease of cotton, coffee bean rot, leaf spot of dieffenbachia and cabbage phomosis (Carter 1973). These cases are characterized by the necessity of feeding by a single insect species allowing greater ingress by a plant pathogen, leading to severe disease, or it was determined that insect feeding in the absence of the plant pathogen is insufficient to cause mortality; furthermore, vectoring by the insect is not necessarily involved. Thus, these findings and a body of scientific work and instructive precedents in weed biocontrol detailed below have created a motivation to explore the effects of insect/pathogen interactions and the contribution they can make to classical weed biocontrol.

THE COMBINED EFFECTS OF INSECTS AND PATHOGENS ON ECOSYSTEMS

As mentioned above, it is a part of every plant pathologists training that whole ecosystems have been altered by the interaction of insects and pathogens. For example, the widespread loss of the American Chestnut, not generally acknowledged as an insect-pathogen interaction, was characterized as such by early workers (Studhalter and Ruggles 1915), who noted the capacity of several insects to carry spores of *Endothia parasitica*, the Chestnut blight fungus and specified two insects that could wound the bark of the American chestnut (*Castanea dentata*) providing avenues for infection by *E. parasitica* (Studhalter and Ruggles 1915). The importance of this interaction has been reinforced by several other such interactions of significant ecological and economic importance, both above- and below-ground: declines of red pine (*Pinus resinosa* Aiton) in Wisconsin and loblolly pine (*Pinus taeda* L.) in the southeast due to root feeding insect and soilborne plant pathogen complexes (Klepzig *et al.* 1991; Eckhardt *et al.* 2007) and such prominent declines of forest tree species as beech bark disease (Mielke *et al.* 1982); ash yellows (Hill and Sinclair 2000), and Dutch elm disease (Agrios 1997). Each of these insect/plant pathogen associations has had profound effects on their hosts over large regions. Such interactions are consistent and powerful in their effects across a range of micro-

environments and ecosystems. Thus, full use of precedents as well as awareness of the ecology of insect/pathogen interactions (and thus the magnitude of their impact, in the case of weed biocontrol, for beneficial effects on ecosystems) would prompt investigation of the utility of exploring for and pre-release testing of insects and pathogens that are mutually associated with the lower density, more frequent disease and reduced thriftiness of invasive species in their native range. Overall, perhaps the closest analogy among all of these insect-pathogen systems to biocontrol of herbaceous perennial is with the declines of pines caused by root-feeding insects and soilborne pathogens described above. These two cases illustrate how similar insect-pathogen interactions can have similar effects in different environments.

SEMINAL WEED BIOCONTROL EFFORTS

Principles for classical weed biocontrol have typically been developed by assessing previous successes and extrapolating a template for how future work should be done. This has been termed “retrospective analysis”. With such analysis, some of the earliest studies could be considered seminal. If past studies can bring evidence for improving biocontrol methodology or fully realizing “best practices”, these studies showed that careful consideration of multiple elements and multitrophic effects are instructive. As described in the report of Dodd (1940), which summed up the research into agents capable of controlling the prickly pear cactus (*Opuntia stricta*), larvae of *Cactoblastis cactorum* were observed to have destroyed prickly pear foliage to the ground. Essentially, soilborne fungi, i.e., not present on the above ground parts were the source of the “disintegration” of the “lower segments”. Disintegration of the lower segments, which can be interpreted as crowns and roots, resulting in stand reductions, so dramatically shown in the pictures from old publications, clearly indicates mortality. Such mortality, based on the present authors findings with leafy spurge and *Fusarium* spp. and *Rhizoctonia solani* (Caesar 2003) and *Lepidium draba* and *Rhizoctonia* spp. (Caesar *et al.* 2010) would be readily attributable to soilborne fungi.

The author’s interpretation of a key passage in the report of Dodd (1940) is that fungi such as *Fusarium* spp. caused the disintegration described:

“The disintegration of the lower segments and butts of *O. inermis* and *O. stricta* during or following a severe attack by *Cactoblastis* would seem to be a physiological breakdown in which various fungi may be contributing factors. Any one of several fungi or any combination of several of these organisms may be isolated from the decaying plants. Apparently, the fungi play a definite role in the destruction, but they do not operate until the plant is supporting or has recently supported a large number of the larvae. These specific diseases are not present in the rotting upper growth, and are not transmitted by the larvae, which very often do not enter the basal portions. It would appear that the plant is weakened by the attack of many larvae, the tissues commence to breakdown, and the fungi are given the opportunity of completing the work of eradication”.

This is supported in principle by others such as Hufaker (1964) who describes a natural enemy as capable of destroying a weed through “1) creating conditions favoring infection by plant pathogens, or 2) by disrupting the competitive advantage of the weed”, in which the biocontrol of the prickly pear cactus in Australia as an example of the former case. If larvae do not normally enter the “butts” (crowns) or roots of the cactus they would not likely carry these fungi, but soilborne pathogens may enter through tissue adjoining the butt or simply infect the crown tissue from soilborne inoculum.

For St John’s wort there is also reason to conclude that its successful control could possibly be attributed to an insect/pathogen interaction. The procumbent growth or rosette stage of this perennial invasive is quite long, and larvae of *Chrysolina* species, the insect releases of which resulted in

successful biocontrol of the weed, do their damage over this period, which would subject the roots and crowns to infection by soilborne pathogens. This is described by Andres and Goeden (1971):

“The synchronized feeding of the adults and larvae of the *Chrysolina* beetle on the basal foliar rosettes of Klamath weed over a long period in the fall, winter and spring deprives the root system of its nourishment. Thus the roots largely disintegrate so that plants cannot secure moisture to survive the long dry summers in California”.

At the very least, feeding damage to the basal foliage could likely affect the crown also and render the roots susceptible to soilborne pathogens. If the roots in fact disintegrate, this would certainly indicate a microbial cause and possibly also include necrotrophic soilborne pathogens such as *Pythium*, *Rhizoctonia* or *Fusarium* spp. Thus, the control of St John's wort may be overlooked as another case of insect-pathogen interaction. In any case, the principle cited here implies that insects that promote greater attack by plant pathogens are worthy of emphasis. Several observations or principles were enumerated in reports made by early workers on insects for the successful biological control of prickly pear cactus (Dodd 1940) and St John's wort (*Hyparrhenia perfoliata*) (Wilson 1943), probably best synthesized in the latter report on work leading to release of the insects which were successful in the control of St John's wort, which included as principle no. 3:

“The effect on the host of an introduced insect is not always limited to the direct damage caused, but is extended if the insect's feeding makes an otherwise immune host susceptible to attack by fungi and bacteria”.

Though one could quibble with precisely how the role of fungi and bacteria are viewed here (foliar plant pathogens were surveyed, but apparently not soilborne pathogens), this principle constitutes a clear recognition in another seminal successful project, in addition to the findings of Dodd that described above of the importance of microbes in weed biocontrol. But more specifically, especially when viewed along with the author's own findings, they support an approach which seeks to exploit for weed biocontrol, insects which stimulate the invasion of highly pathogenic soilborne fungi. Furthermore, principle no. 8 in Wilson (1943) in emphasizing a likely greater effectiveness of specialized insects as due among other reasons, to “the decay occurring in the host from the development of bacteria and fungi in the insect's frass”. Collectively, in both the early descriptions of the control of prickly pear cactus and St John's wort and those of later authors who summarized the work in various biological control texts, soilborne pathogens are not specifically mentioned, but descriptions of disease development are indicative of them (Holloway and Huffaker 1951; Huffaker 1964; Andres and Goeden 1971; Crafts 1975). Interestingly, the various tenets of biological weed control covering such ideas as reduced competitiveness of a target weed due to even minor damage by an insect, the need for host specificity of an agent, criteria for pre-release studies, climate-matching, are nearly all adhered to today. The exceptions have been those principles in which fungi and bacteria are a consideration. This author, when recalling these various precepts, has failed to see how recently extolled “best practices” for classical biocontrol of invasive weed programs can escape being considered as “reinventing the wheel”. What would be a novel best practice among those usually discussed is the re-inclusion of consideration of plant pathogenic synergists in how a “campaign” against a new target invasive weed is conducted. Pre-release studies would include investigation into the nature of interactions, being chiefly based on identifying soilborne pathogens associated with damage to roots and crowns by larvae of natural enemies. This author has noticed that insect/plant pathogen interactions are often treated as tenuous or fragile or too easily affected by other ecological factors. That they are instead durable and consistent is the point of the following sections.

FINDINGS IN SUPPORT OF THE NEED FOR INSECT/PATHOGEN SYNERGISMS

Research within weed biocontrol

Despite consideration in seminal projects for a microbial role in mechanisms by which insects were successful in classical biological control of perennial invasive weeds, this topic was ignored for years. It was revived by work done in relation to water hyacinth (Charudattan *et al.* 1978), describing how a fungal pathogen, *Acremonium zonatum* predominated in isolations from insect-damaged plants. Rather than a vector relationship between insect and pathogen, the insect damage created avenues of ingress by *A. zonatum* and other plant pathogens. The relationship between larval feeding of natural enemies of water hyacinth and the presence of *A. zonatum* or *Acremonium* spp. has been described from other locations (Sanders 1982; Galbraith 1987; Evans and Reeder 2001; Hernández *et al.* 2007). In addition, *Acremonium* spp. have also been found at other locations where there was no mention of association with insects (Rahim and Tawfig 1984; Jiménez and Charudattan 1998). It has been supposed that the nature of the insect/pathogen relationship(s) of water hyacinth is exceptional. However, there is increasing evidence that similar insect/pathogen relationships exist. The finding by Charudattan *et al.* (1978) that a synergistic insect/pathogen combination leads to mortality under controlled conditions has been shown with regard to another insect-pathogen combination: that of *Rhizoctonia solani*, *Fusarium oxysporum* and *Aphthona* spp. against leafy spurge (Caesar 2003). Another similarity of the leafy spurge work to that of water hyacinth was that there were complexes of plant pathogens associated with insect damage, which occurred over a wide geographic range (Caesar *et al.* 1993; Caesar 1994a, 1994b, 1996; Caesar *et al.* 1998; Nash 1998; Caesar *et al.* 1999; Caesar and Lartey 2008). Comparison of results of the pioneering work of Charudattan *et al.* (1978) on fungi associated with insect damage to water hyacinth to later work by others on the same topic (Evans and Reeder 2001; Hernández *et al.* 2007) indicates that 4 genera with confirmed pathogenicity (or containing plant pathogenic species) were isolated from this host at each of the three localities encompassed by this work (Table 1). Further, there were at least 8 species associated with tunnels made by larvae of insects, based on work in Florida and Argentina (Table 1). It has been concluded that the most exciting and promising use of pathogens of water hyacinth is in combination with insects (Evans and

Table 1 Association of pathogenic (or genera with pathogenic species) fungi with insect-infested tissue of water hyacinth and their further association with tunneling of host tissue by larvae of insect natural enemies.

Identification	Origin	Association with larval tunneling ^a
<i>Acremonium</i> spp.	Upper Amazon	not determined
	Florida	X
	Argentina	X
<i>Alternaria</i> spp.	Upper Amazon	not determined
	Florida	X
	Argentina	X
<i>Cladosporium</i> spp.	Florida	X
	Argentina	X
<i>Curvularia</i> spp.	Upper Amazon	not determined
	Florida	X
	Argentina	X
<i>Fusarium</i> spp.	Upper Amazon	not determined
	Florida	X
	Argentina	X
<i>Mycosphaerella</i> spp.	Florida	X
	Argentina	X
<i>Pestalotia/pestalotiopsis</i>	Florida	X
	Argentina	X

^aThe study done in the upper Amazon does not specify larval tunneling, referring to the occurrence of insect damage alone.

Reeder 2001). Given the similarity of that system in the several respects presented above to terrestrial invasive weeds such as leafy spurge, the prospects for the use of plant pathogens in combination with insects that damage tissue in these situations should be regarded as similarly promising. Conversely, the degree of how promising an insect agent is should be based on how likely it is to stimulate invasion of host tissue (preferably roots) by plant pathogens. If the large amount of work that drew upon the Charudattan *et al.* (1978) study ultimately led, as in a recent study (Jiménez and Gómez Balandra 2007), to implementation of an insect/pathogen strategy which was successful in reducing water hyacinth density, it bears out the above endorsement by Evans and Reeder of such an approach and should support a similar approach to controlling several terrestrial invasive weeds such as deep-rooted herbaceous perennial species.

The importance of the soil microbiota in affecting plant community structure

A long course of research on how soil microbes affect plant communities, and how plant communities alter the soil microbial community has established the importance of these mutual effects (Ehrenfeld *et al.* 2005). The applicability of the resulting body of knowledge to the issue of the invasive plant ecology has also been developed and recognized (Ehrenfeld *et al.* 2005), probably beginning with studies by Van der Putten (Van der Putten *et al.* 1988) and Bever (1994a). Some of these themes have previously been discussed from a weed biocontrol perspective by the present author (Caesar 2005). Additionally, the effects of below-ground herbivory by insects and nematodes on plant communities have received increased emphasis in studies conducted in the 10-15 years prior to the writing of this essay. Much of this work on root herbivore/soil microbe interactions was focused on nematodes and the whole soil microbial community or fungi isolated from root pieces on a general medium without apparent regard, for example, to root lesions (De-Rooij-van der Goes *et al.* 1995). When more exacting phytopathological methods were applied in feedback work, root herbivores were not part of the study (Bever 1994; Mills and Bever 1998). Thus, because of the aims of these studies, seminal in many ways, a focus on herbivory and the application of classical plant pathological methods (isolation from diseased tissue, use of selective along with general media for isolations and completion of Koch's postulates) did not occur at the same time. Van der Putten (1988) first drew a conceptual parallel between agricultural systems in which replant diseases due to biotically-based plant-soil negative feedbacks (Bever 1994) occurred on horticultural crops and natural systems in which a decline or failure of establishment of dune grasses occurred. Initially, a somewhat coequal partnership between plant parasitic nematodes and plant pathogens was supposed for the failure to establish or the decline of the clonally spreading dune grass *Ammophila arenaria* (Van der Putten and Troelstra 1990). Eventually, the effect of the free-living plant parasitic and plant endo-parasitic nematodes alone on the biomass of the dune grasses in this system was determined to be minor at best (Verschoor *et al.* 2002; Brinkman *et al.* 2005b; Van der Stoel *et al.* 2006). The effects on dune grass biomass have been attributed to complexes of fungi or the whole soil community (Brinkman *et al.* 2005b). The present author, unaware of the analogy drawn by Van der Putten between replant diseases and negative plant-soil negative feedback, made a similar comparison recently (Caesar 2005). Upon further consideration, however it can be concluded that the analogy is only partial. The comparison of the dune grass feedback with a replant or sick soil syndrome can only properly be made when attempts to isolate one or more plant pathogens have been unsuccessful. Negative feedback and replant or sick soil disease both describe a syndrome in which the rhizosphere microflora becomes altered from one generally benign to generally negative toward a given plant

species. The existence of weak or "minor pathogens" in agronomic systems (Salt 1979) would certainly support the analogy made by Van der Putten (1988). Further, the work of Van der Putten and coworkers extends to natural systems the concept that negative feedback or replant disease can have important effects on plant growth, especially grasses. However, that one or more overt plant pathogens may be the cause of the dune grass negative feedback cannot be precluded based on how the dune grass feedback studies were done. Planting of seedlings and cuttings of the dune grass into rhizosphere soil from a well established stand of *A. arenaria* led to high mortality in an outdoor experiment (Van der Putten *et al.* 1988), indicating severe disease. No isolations from the brown, stunted roots were done. The methodology applied in this work may have obscured the importance of overt pathogens in combination with herbivores (nematodes), which were found to be present in low numbers. The effects of nematodes were only additive when tested along with a complex of fungi isolated from the rhizosphere that individually had little negative effect on the dune grass. The low populations of nematodes noted in the dune grass studies (Van der Putten and Troelstra 1990; De-Rooij-van der Goes *et al.* 1995; van der Stoel *et al.* 2002) do not eliminate them as participants in a synergism because root damage by pathogenic fungi can prevent completion of the life cycles of endoparasitic nematodes (Back *et al.* 2002). Also, that *Meloidogyne maritima* was the sole species of nematode (combinations of *M. maritima* with two other species, *Heterodera* and *Pratylenchus penetrans* did not affect biomass (Brinkman *et al.* 2005b)) which restricted biomass of the dune grass in field studies (Brinkman *et al.* 2005a) is interesting. *Meloidogyne* species are documented to be synergistic with such soilborne pathogens as *Rhizoctonia*, *Fusarium* and *Phytophthora* spp. (Powell 1971), all of which require selective media and some experience to successfully isolate from host plants. Thus, advancing knowledge of any involvement of plant pathogens in negative effects of root herbivore/soil microbial community on the dune grasses may lie in applying standard plant pathology methods and Koch's postulates to determine what if any soilborne pathogens are associated with root herbivory by nematodes in coastal dune grass stands. This would require isolation from roots. Overall, despite some ambiguities from a plant pathological point of view, this body of feedback work establishes that the soil microbial community can alter plant succession and community structure and that the effect of the nematode herbivory alone was minor at best. This is similar to a study by the author, which showed that soilborne pathogens were ca 2.5 times more likely to be the cause of weed mortality than a root-feeding insect (Caesar 2003). The dune grass feedback studies further showed that root herbivores, soilborne fungi (and probably some key soilborne plant pathogenic fungal species, as yet unidentified, but likely including *Fusarium* spp. *Pythiaceae* spp. and possibly *Rhizoctonia* spp.), constitute negative feedback. Rather than a renewed or closer focus on applying plant pathological methodology including quantifying the inoculum levels in the field to the various systems, much work on negative feedback and invasive weeds now focuses on mycorrhizae. Several field studies that have assessed the comparative effects of plant pathogens and mycorrhizae on plant communities have shown that plant pathogens override mycorrhizae (Hetrick and Wilson 1991; Bever 1994; Holah and Alexander 1999; Blomqvist *et al.* 2000; Olf *et al.* 2000; Šmilauer and Šmilauerova 2000; Kardol *et al.* 2007). Invasion biology is now driven by studies in the field of ecology. Few have specifically addressed the combined effects of insect root herbivory and the soil and rhizosphere microbial community, especially plant pathogens, despite how common such effects are in forest and agroecosystems. Pursuit of a plant pathological approach has important implications. The interaction of insect or nematode herbivory and plant pathogens has been shown to cause accelerated mortality of a target invasive aquatic (Charudattan *et al.* 1978) and terrestrial (Caesar 2003) weed.

In the latter situation root herbivory would result in a higher inoculum potential of soilborne pathogens as an accelerated form of negative plant-soil feedback, it has been hypothesized (Caesar 2005). This biotic legacy of accelerated negative feedback would result in higher inoculum loads of soilborne pathogens that would in turn negatively affect attempts to restore native species following successful biological control. Studies currently being conducted by the author and collaborators confirm this theory. In restoration plots in areas from which leafy spurge has been dramatically reduced by biological control effected by insect/pathogen interactions, there is significant mortality of forbs transplanted into the plots, from which soilborne pathogens are isolated at a high frequency (Caesar *et al.* 2008).

CONCLUSIONS

Including consideration of direct plant pathogen/insect interactions from the very outset of programs is called for by precedent set at the very beginning of successful large scale invasive plant biocontrol (Dodd 1940; Wilson 1943). It is also supported by work begun in the late 1970's (Charudattan *et al.* 1978) that has resulted in recent success (Jiménez and Gómez Balandra 2007) and newer findings that may aid achievement of a greater percentage of successfully impacted infestations of a perennial weed such as leafy spurge. Such interactions and the microorganisms involved, if discovered to be associated with effects of a candidate insect in the native range of a weed, such as *Lepidium draba*, should be part of pre-release assessment of the agent along with host range testing and other matters. Studies by the present author indicate that there are plant pathogens associated with root herbivory on several invasive species and that the effects of these plant pathogens can display ranges of virulence from moderate to severe stunting to mortality of the host. Plant-soil negative feedback, established in the literature in the last several years, now through the present authors' work includes the dimension of herbivore x minor or major pathogen interaction as essentially an accelerated form of negative feedback. Thus, given the well-documented phenomenon of negative plant-soil feedback in natural systems, perhaps initial releases of insects might best be targeted toward the oldest weed infestations, whereby the optimum effects of both direct and indirect interactions between herbivores and plant pathogens or deleterious bacteria can be realized.

Combinations of an insect and a plant pathogen or pathogens per se are not intrinsically valuable or compelling. Some combinations by their very nature, however they may serve to present a concept (Campanella *et al.* 2009), are not compelling for weed biocontrol without a causative link, established from fieldwork in the native range, between the sparseness of smaller stands seen in the native range of a weed compared to the invaded range and an insect/pathogen interaction. Such field studies (Caesar 2006) can and should be done. Whatever the impacts of interactions which do not involve direct interaction between an herbivore creating an avenue for ingress by soilborne plant pathogens, this essay is meant to highlight the potential value of emphasizing root-attacking agents especially against herbaceous perennial weeds. Clearly there are instances where an insect, without evidence of any pathogen involvement, is a key to causing plant mortality and thus highly effective control of a target invasive, such as diffuse knapweed (Myers *et al.* 2009). For invasive species such as *Lepidium draba* however, an herbaceous, aggressively spreading perennial, ca. 75% of the biomass is below ground (Miller *et al.* 1994). Logically, priority should be given to root-attacking agents (which quite certainly would invoke interactions with soilborne plant pathogens) before as many as 12 foliar herbivores, as in the case of diffuse knapweed, are released.

Thus, several different lines of research constitute a rationale for the inclusion of soilborne plant pathogens as a factor in research leading to the introduction of classical

insect biological control agents against at least perennial herbaceous invasive weeds. They are: the common occurrence of direct insect/pathogen interactions in diseases of economic crops, the ability of such interactions to cause widespread effects on ecosystems, the precedents in which insect/pathogen interactions have been documented as related to the success of weed biocontrol, and recent findings. Consideration at least and application at best of such information would certainly strengthen best practices in classical weed biocontrol. That negative plant soil feedback may actually be caused primarily by plant pathogens indicates that the best sites for initial releases of insects for weed biocontrol may be older stands where the feedback (accumulation of pathogen inoculum) has developed. The involvement of plant pathogens in synergisms with insects indicates that supplementation of insect releases with plant pathogens may be necessary where establishment of the insects has failed to lower stand density of the target weed. Insect/pathogen interactions as a mechanism for achieving biocontrol of perennial weeds resulting in increased inoculum levels of soilborne pathogens (Caesar 2003) would be predictive of significant disease affecting the restoration of native forbs. Such has been found to occur (Caesar *et al.* 2009). Thus, the extent of the role of insect/plant pathogen interactions in weed biocontrol described herein indicates that plant pathogens and plant pathology are unique in being important before, during and after weed biocontrol.

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