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

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 flavescens* had been released 4 years prior, found that the stand density was dramatically decreased. The stand showed the dramatic effects of having been “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 shots of spurge that were wilted or dead. The roots of such plants were damaged by larvae of *A. flava*. The samples collected included *Rhizoctonia solani*, *Fusarium spp.*, 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*, *Chamaesphecia* or *Oberesa* 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 synergies are an integral part of their undergraduate and graduate studies. The nature and extent of 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, liniot of cotton, internal boll disease of cotton, coffee bean root, leaf spot of dientenbachia 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; as h yellows (Hill and Sinclair 2000), 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), which summed up the research into agents capable of controlling the prickly pear cactus (*Opuntia stricta*), larvae of *Cactoblastis cactorum* 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 the disease may be isolated from these 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 rotted 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 tissue commences to breakdown, and the fungi are given the opportunity of completing the work of eradication”.

This is supported in principle by others such as Hufnäker (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.

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* would be readily attributable to soilborne fungi. This is supported in principle by others such as Hufnäker (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 work 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 *Chrysoryla* species, the insect releases of which resulted in environment and ecosystem.**
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 Chrysomela beetle on the basal foliage of Klamath weed over a long period in the fall, winter and spring deprives the root system of its nourishment. Thus the roots large in the late spring 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 insects’ 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 adapted to today. The exceptions have been those princi- ples 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. Prerelease 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 SYNERGISM**

**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>
<thead>
<tr>
<th>Identification</th>
<th>Origin</th>
<th>Association with larval tunneling</th>
</tr>
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<tbody>
<tr>
<td><em>Acremonium</em></td>
<td>Upper Amazon</td>
<td>not determined</td>
</tr>
<tr>
<td><em>Alternaria</em></td>
<td>Argentina</td>
<td>X</td>
</tr>
<tr>
<td><em>Cladosporium</em></td>
<td>Florida</td>
<td>X</td>
</tr>
<tr>
<td><em>Curvularia</em></td>
<td>Upper Amazon</td>
<td>not determined</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>Upper Amazon</td>
<td>not determined</td>
</tr>
<tr>
<td><em>Mycosphaeria</em></td>
<td>Florida</td>
<td>X</td>
</tr>
<tr>
<td><em>Pestalotia</em></td>
<td>Florida</td>
<td>X</td>
</tr>
<tr>
<td><em>Pestalotiopsis</em></td>
<td>Arge atina</td>
<td>X</td>
</tr>
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*The study done in the upper Amazon does not specify larval tunneling, referring to the occurrence of insect damage alone.*

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**Table 1** Association of pathogenic (or genera with pathogenic species) fungi with insect-infested tissue of water hyacinth and the further association with tunneling of host tissue by larvae of insect natural enemies.
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 amount of work that grew upon the Chittenden et al. (1978) study ultimately led, as in a recent study (Jimenez and Gomez 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 microbiota in affecting plant successfully. Thus, because of the aims of these studies, seminai 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 feedbacks due to the combined effects of insect herbivory and 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 fungal 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. Pythium spp. and possibly Rhizoctonia spp.), constitute negative feedback. Rather than a renewed or closer focus on applying plant pathological methodology including quantitative field protocols, most recent work 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 (Hietrick and Wilson 1991; Bevers 1994; Holah and Alexander 1999; Blomqvist et al. 2000; Olff et al. 2000; Smilauer and Smilauerova 2000; Kusin et al. 2007). However, the role of negative feedback in the biology of nutrient cycling and nutrient availability 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 (Churadattan 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 (Charrudattan et al. 1978) that has resulted in recent success (Jiménez and Gómez Balandra 2007) and newer findings that may support development of a new concept. The role of successful 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, 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 having 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 strengthen best practices for 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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