Can Competition Experiments Be Used to Evaluate the Potential Efficacy of New Water Hyacinth Biological Control Agents?

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Abstract

Two factors are of concern when considering a new biological control agent for introduction. The first is the safety of the organism (i.e. its host specificity) and the second is the potential for the organism to control the target weed (i.e. its efficacy). Methods for evaluating safety before introduction are well known but scant attention has been paid to pre-release evaluation of the efficacy of the candidate organisms. This is understandable inasmuch as the agent's performance depends on the presence or absence of density-dependent population regulating factors that will differ between the donor area and the recipient country. However, this is of less concern when the agent has already been introduced elsewhere, where it can be studied without the influence of density-dependent regulators. Experiments comparing the effectiveness of the new agent with that of another, more widely known agent, can then be used to determine the relative value of the former with the known impacts of the latter. Additive series analysis (inverse linear models) of competition between water hyacinth and water lettuce as mediated by herbivory has been suggested as a means of judging the relative value of new agents. This approach is fraught with difficulties inasmuch as there will always be unknown factors that affect the abundance of new agents (i.e. biotic resistance), but it could enable assessment of the *potential* value of the proposed introduction and, in so doing, perhaps pre-empt the introduction of risky agents that provide little control value.

CLASSICAL biological control of a pestiferous non-native plant involves the deliberate introduction of plant-feeding insects, mites, or phytopathogens (collectively called biological control agents, or herein, bioagents) from foreign sources to provide previously missing density-dependent regulation of the pest species in its adventive range. Typically, the bioagent is derived from within the native range of the pest and introduced into a new area where control is needed. The safety of the introduced organism is of utmost concern inasmuch as economically or ecologically important non-target plant species in the recipient region may be at risk, and this risk escalates as more and more agents are introduced. Thus, it is essential to introduce the least number of species needed to provide the control needed. In order to minimise the number of introductions, it would be useful to determine beforehand which species, from among the cadre of potential bioagents available, would be the most effective. While this is often called for (Harris 1973), it is seldom done.

Techniques for determining the safety of a bioagent, in terms of its fidelity towards the use of the target plant, consist mainly of bioassays of host specificity. These 'host-specificity tests' have a long history of use and are very predictive (Pemberton 2000).

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However, standardised methods for evaluating the potential impact of candidate bioagents are lacking. Harris (1973) and later Goeden (1983) attempted to develop a scoring system based on specific attributes of the candidate agents. Both systems emphasised the amount of damage done to the plant on a per insect basis or the capacity for population growth of the agent. Unfortunately, these tended to be 'one size fits all' and failed to take into account the uniqueness of each weed–insect association. As a result, while they do provide 'rule-of-thumb' guidelines, these scoring systems are otherwise of limited usefulness. Among other things, they fail to consider compensatory abilities and complementary characteristics of the various target plants, which render them vulnerable (or not) to damaging effects of the agents. In other words, in order to be effective, the damage done by the agent must be directed towards the invasive attributes of the plant that enable it to dominate, so a useful scoring system must be tailored to each weed target. This is difficult, at best, especially when, at the outset of a project, so little is usually known about these agents and the target plant.

There are few alternatives for directly assessing the value of a new, previously unused bioagent. Any such appraisal must mimic a true biological control scenario in which the population increase of the agent is not limited by density-dependent regulators, thus enabling their populations to attain greater densities than those normally found in the native environment. Such assessments, which are best done under natural circumstances, may be difficult to accomplish in the native range of the bioagent, because of the presence of density-dependent regulators that pre-empt buildup of the bioagent's population and therefore fail to simulate a true introduction scenario involving hyper-abundant bioagent populations. Furthermore, any such assessment must be sensitive to subtle effects of the bioagents, so as not to disqualify those that might provide important, long-term effectiveness.

We propose direct experimentation to provide data on the relative value of one agent compared to another. This does not resolve the difficulties involved in doing the studies in the native area, but this approach is quite possible when the agent has been previously introduced elsewhere and is being considered for introduction into a new area. The mirid bug *Eccritotarsus catarinensis* provides a useful example. It was first introduced into South Africa and is being considered for introduction into North America. Laboratory-based host-specificity testing showed that it fed and developed on pickerelweed, a valued North American native plant. Follow-up field studies in South Africa revealed that, while it might spill-over to pickerelweed when adjacent to heavily infested water hyacinth mats, it did little damage and did not colonise isolated pickerelweed stands (Hill et al. 2000). Thus, it seems as though this agent might, in fact, be safe to release in North America. However, the host-specificity data clearly indicate that there is some risk to pickerelweed. Considering that pickerelweed is severely damaged by drifting water hyacinth mats as well as by herbicidal control operations directed against water hyacinth, this risk might be worth taking. The decision to release the mirid must therefore weigh the potential damage to pickerelweed against the benefit that it might provide. However, the effectiveness of the mirid is not yet known. We are proposing to compare the effects of the mirid with the effects of the better-known bioagent, the weevil *Neochetina eichhorniae*, on the competitive relationship between water hyacinth and water lettuce. In so doing, we hope to determine whether the mirid would be more or less effective than the weevil and to quantify the difference.

The effects of the mirid are likely very subtle. It is a quite small insect that causes little damage per individual, which is neither overt nor easily quantified. It feeds on leaf surfaces by sucking plant juices, creating brownish patches that vary in extent and intensity (similar to spider mite damage). While this damage may be debilitating to some degree, it does not seem lethal. In situations such as this, competition studies may be able to detect these subtle effects by measuring the reduction of the plant's competitive ability against another aggressive species. Pantone et al. (1989) proposed the use of additive series experiments analysed using inverse linear models to evaluate the efficacy of bioagents before release (although they did not address the aforementioned difficulties in doing these studies in the agent's native range). They further demonstrated the utility of the method by detecting the effects of a nematode on competition between the fiddleneck weed and wheat. It thus occurred to us that this approach might be useful for determining the value of the water hyacinth mirid. We have used this model previously (Van et al. 1998, 1999) to compare the influence of two hydrilla biological control agents and to investigate the effect of soil fertility on competition between the two aquatic plants *Hydrilla verticillata* and *Vallisneria americana*.

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Additive Series Competition Experiments and the Inverse Linear Model

Pantone et al. (1989) provided a thorough explanation of additive series competition experiments and the application of the inverse linear model. Their paper should be consulted for details. Competition experiments involve planting mixtures of two plant species and, after a period of growth, measuring yield components of each species and comparing them between species. Additive series competition experiments differ from replacement series competition experiments in that the total number of plants used for the two species varies as the mixture ratio increases (i.e. 3 of species A vs. 0 of B for 3 total; 3 of A vs. 3 of B for 6 total; 3 of A vs. 6 of B, or 9 total; etc.). In contrast, replacement series experiments use a constant total number of plants while the ratio of the two varies. Pantone et al. (1989) used the mixtures given in Table 1 in their additive series experiments

Table 1. The additive series planting ratios of wheat and fiddleneck used by Pantone et al. (1989)

Wheat	Fiddleneck			
	:0	:20	:80	:160
0:		0:20	0:80	0:160
20:	20:0	20:20	20:80	20:160
80:	80:0	80:20	80:80	80:160
160:	160:0	160:20	160:80	160:160

A control series was planted without nematodes and a duplicate second series (treatment) was planted and the plots were inoculated with 10^6 fiddleneck gall nematodes (*Anguina amsinckiae*). Plants were harvested after 5–6 months and average yield per plant (Y) was measured in terms of shoot dry weight, seed number, and total seed biomass per plant.

Data were analysed using multiple linear regressions of the inverse of the yield component as the dependent variable and the planting density of wheat and fiddleneck as two independent variables as such:

$$1/Y_f = a_{fo} + a_{ff}d_f + a_{fw}d_w$$

$$1/Y_w = a_{wo} + a_{ww}d_w + a_{wf}d_f$$

Here Y_f is the average yield per plant for fiddleneck, Y_w is the average yield per plant for wheat, d_f is the planting density of fiddleneck, and d_w is the planting density for wheat. The coefficients a_{ff} and a_{ww} measure intraspecific competition of fiddleneck and wheat, respectively. The coefficient a_{fw} measures the interspecific effect of wheat on fiddleneck yield, and the coefficient a_{wf} measures the interspecific competitive effect of fiddleneck on wheat yield. The ratio a_{ff}/a_{fw} measures the effects of intraspecific competition of fiddleneck on itself relative to the interspecific competition of wheat on fiddleneck. In other words, it equates the competitive effect of a single fiddleneck plant with the number of wheat plants that would be expected to have an equivalent effect on fiddleneck yield (i.e. it takes x number of wheat plants to produce the same effect as a single fiddleneck plant on fiddleneck yield). Likewise, the ratio a_{ww}/a_{wf} measures the effect of wheat on wheat yield relative to the effect of fiddleneck on wheat yield. The data can be graphically analysed as a 3-dimensional surface response plane for each plant species in which the slope in one direction represents the effect of the species own density upon its yield (intraspecific competition) and the slope in the other direction represents the effect of the competing species (interspecific competition). It must be borne in mind that, because the inverse of the dependent variable is used, a higher value represents a lower yield. Likewise, a steep slope represents a strongly reduced yield in response to increasing plant density. Results of one of the experiments conducted by Pantone et al. (1989) are presented in Table 2. Note that increasing fiddleneck density strongly reduced fiddleneck yield per plant, as evidenced by the steep slope reflected in the coefficient a_{ff}, when nematodes were absent. However, the effect of wheat on fiddleneck yield per plant was slight. The ratio of the two values (a_{ff}/a_{fw}) indicates that the effect on fiddleneck yield of increasing the density of fiddleneck by a single plant was equivalent to increasing the density of wheat by 33 plants. When nematodes were present, however, the effects of the two species were similar, as reflected by the ratio of the two coefficients being near unity.

The complementary analysis similarly indicates that the interspecific effect of fiddleneck on wheat yield was much greater than the intraspecific effect of wheat on itself. Increasing the density of wheat by one plant had the equivalent effect on wheat yield of adding 0.3 fiddleneck plants. When nematodes were present this increased to 0.72 fiddleneck plants.

Recently, similar experiments have been done in Florida (Van, unpublished data) to examine the effects of the weevil *N. eichhorniae* on competition between water hyacinth and water lettuce (*Pistia*

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stratiotes) (Table 3). In this example, without weevils, increasing the density of water hyacinth by one plant produced 18.5 times the effect on water hyacinth yield of increasing the water lettuce density by one plant. In other words, it required nearly 20 water lettuce plants to produce the equivalent effect of a single water hyacinth plant on water hyacinth yield. With weevils in the system, however, water hyacinth remained the superior competitor but its advantage was reduced to less than 2 to 1. Likewise, without weevils an increase in water lettuce density of one plant affected water lettuce yield by an amount equivalent to only 0.15 water hyacinth plants (or 7 water lettuce plants were required to produce the effect of 1 water hyacinth plant) but with weevils present this ratio increased to nearly unity.

Clearly, these analyses provide a useful way of assessing the impact of a bioagent on two-species competition, but can they be used to compare bioagents? The studies by Van et al. (1998) indicate that they can. They compared two hydrilla control agents in terms of their effects on competition between *H. verticillata* and *V. americana*. They showed that, in the absence of bioagents, intraspecific competition by *Hydrilla* on itself was 8.3 times stronger than interspecific competition from *Vallisneria*. In the presence of the leaf-mining fly, *Hydrellia pakistanae*, however, intraspecific and interspecific effects were nearly equal (a_{hh}/a_{hv} = 1.3). The weevil *Bagous hydrillae* produced a much smaller shift in the competitive balance (a_{hh}/a_{hv} = 7.6), which was not much different from the control. As a result, one might conclude that the fly is nearly six times better than the weevil, in terms of its ability to alter the competitive balance between these two plant species.

Given the positive results of these studies, we are now comparing the two species of *Neochetina* (*N. eichhorniae* vs. *N. bruchi*) in terms of their ability to alter the competitive relationship between water lettuce and water hyacinth. The results are not yet in. This experiment involves 96 experimental units (8 planting densities × 4 insect levels × 3 replicates). The 8 planting densities (the minimum necessary) encompass factorial combinations of 0, 3, or 9 water hyacinth and water lettuce plants (minus the 0:0 combination). The insect treatments consist of *N. eichhorniae* alone, *N. bruchi* alone, both species together, or neither species (as a control).

The logistics of setting up such a large experiment have been difficult. Nevertheless, if this experiment produces useful results, we are planning a similar experiment to be conducted in South Africa to

Table 2. Regression coefficients from analyses of the effects of nematodes and plant density on reciprocals of the biomass yields of wheat or fiddleneck (from Pantone et al. 1989)

Plant	Treatment	Regression coefficients					
		a_{ff}	a_{fw}	a_{ff}/a_{fw}	a_{ww}	a_{wf}	a_{ww}/a_{wf}
Fiddleneck	Control	8.24	0.25	33.0			
	Nematode	8.76	8.40	1.04			
Wheat	Control				4.97	16.4	0.30
	Nematode				5.81	8.09	0.72

Table 3. Regression coefficients from multiple regression analyses of the impacts of weevils and plant density on the reciprocal biomass yield of water hyacinth and water lettuce (from Van, unpublished data)

Plant	Treatment	Regression coefficients (× 10^{-3})					
		a_{ww}	a_{wl}	a_{ww}/a_{wl}	a_{ll}	a_{lw}	a_{ll}/a_{lw}
Water hyacinth	Control	0.943	0.051	18.5			
	Weevil	3.72	2.28	1.63			
Water lettuce	Control				9.41	62.1	0.15
	Weevil				3.24	3.52	0.92

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compare the mirid with *N. eichhorniae*. In so doing, we hope to quantify the effect of the mirid relative to the effect of the weevils using the weevil as a standard. However, this involves another difficulty: how to determine the numbers of each insect species to be used when two very different plant-feeding insects are involved. In the case of the two *Neochetina* species, this is not a problem. Both are about the same size and produce the same type of damage. However, comparing the chewing damage of the larger weevils with the sap-sucking damage of the tiny mirid is another matter. Is it appropriate to merely use the same number of each species, despite the size difference and the disparity in the type and amount of damage? Would it be better to introduce equivalent weights of both species? Obviously, it would be best to use a range of infestation levels of each insect to measure the densities of each needed to produce equivalent effects, but the size of the experiment then becomes prohibitive. These and many other questions must be resolved before proceeding with plans for this experiment.

It is important to keep the limitations of these experiments in mind. First, cages are used and several types of cage effects could lead to erroneous conclusions. Secondly, the experiments described above include only a few of the multitude of environmental parameters that might affect the outcome of competition. The effects of the insects might be compromised, for example, by high or low nutrients, but incorporation of a nutrient treatment in the experiment design would at least double the size to 192 experimental units in the case of the two-weevil experiment described above. Thus, while it is important, if possible, to retain the full additive series so as to produce comparable regression coefficients, it might not be possible to answer all pertinent questions in this manner. We are therefore considering additional experiments with varying nutrient levels but fixed combinations of the two plant species for comparisons between *N. eichhorniae* and *N. bruchi.* This is less desirable, but much more practical.

References

Goeden, R.D. 1983. Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. Protection Ecology, 5, 287–301.

Harris, P. 1973. The selection of effective agents for the biological control of weeds. The Canadian Entomologist, 105, 1495–1503.

Hill, M.P., Center, T.D., Stanley, J., Cordo, H.A., Coetzee, J. and Byrne, M. 2000. Host selection by the water hyacinth mirid, *Eccritotarsus catarinensis*, on water hyacinth and pickerelweed: a comparison of laboratory and field results. In: N.R. Spencer, ed., Proceedings of the 10th International Symposium on Biological Control of Weeds, 4–9 July 1999, Bozeman, Montana, 357–366.

Pantone, D.J., Williams W.A. and Maggenti, A.R. 1989. An alternative approach for evaluating the efficacy of potential biocontrol agents. 1. Inverse linear model. Weed Science, 37, 771–777.

Pemberton, R.W. 2000. Predictable risk to native plants in weed biological control. Oecologia, 125, 489–494.

Van, T.K., Wheeler, G.S. and Center, T.D. 1998. Competitive interactions between hydrilla (*Hydrilla verticillata*) and vallisneria (*Vallisneria americana*) as influenced by insect herbivory. Biological Control, 11, 185–192.

— 1999. Competition between *Hydrilla verticillata* and *Vallisneria americana* as influenced by soil fertility. Aquatic Botany, 62, 225–233.