LCMR Research Addendum
For proposed research on:

BIOLOGICAL CONTROL OF EUROPEAN BUCKTHORN

(Revised from 2003 LCMR Research Addendum)

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ABSTRACT

Common buckthorn (*Rhamnus cathartica*) and glossy buckthorn (*Rhamnus frangula*) are two introduced Eurasian buckthorn species that are invading a number of habitat types in the northeast and north central part of the United States and Canada. Both species are very adaptable, forming dense thickets that shade out the ground, inhibiting the growth of native forbs, shrubs, and tree seedling. Eurasian buckthorns are very difficult to manage once they become established. An assortment of labor intensive management methods have been attempted with varying success. Conventional buckthorn management typically provides only short-term control. Buckthorn easily regenerates from roots or new plants establish from the seed bank.

A long-term solution to buckthorn management could be biological controls, but there has not been sufficient research to determine its potential. Initial research conducted by Center for Applied Bioscience-Switzerland for the Minnesota DNR suggests at least 12 insects that should be evaluated for potential biological control in future research. The goals of the research are to study the distribution of potential natural enemies of *Rhamnus* spp in Europe,(i.e. to locate and further collect potential natural enemies of *Rhamnus cathartica* and *Frangula alnus*, carry out host specificity studies on potential control agents and finally to survey buckthorn in Minnesota to determine if any native or non-native insect species are currently found on buckthorn or cause damage to buckthorn in.

Problem Identification/Background

Common buckthorn (*Rhamnus cathartica*) and glossy buckthorn (*Rhamnus frangula*) are two introduced Eurasian buckthorn species that are invading a number of habitat types in the northeast and north central part of the United States and Canada. Common buckthorn invades woodlands, prairies and roadsides, while glossy buckthorn generally invades wet areas including wetlands, fens, bogs and riverbanks (Randall and Marinelli 1996, Wisconsin DNR 1997, White et al. 1993). Both species are very adaptable, forming dense thickets that shade out the ground, inhibiting the growth of native forbs, shrubs, and tree seedlings (Heidorn 1991, Randall and Marinelli 1996).

Some wildlife food sources are eliminated when buckthorn displaces native plant species such as white oak. The oaks, for example, are utilized for food by 186 species of birds and mammals. And the value of oaks to traditional game species such as wood ducks, wild turkey, black bears, and deer is widely recognized (Pfannmuller 1991). Fall migrants such as thrushes, tanagers, vireos and warblers require fatty fruits for their migration. Exotic shrubs such as buckthorn are less likely to provide the proper nutrients and deliver them when the native migratory birds need them (Kress unknown date). They have also been linked to increased predation in songbird populations (Schmidt and Whelan 1999).

Common and glossy buckthorn are also alternate hosts for the fungus (*Puccinia coronata*) that causes oat rust disease (Harder and Chong 1983). Eurasian buckthorn is also an overwintering host for the invasive Asian soybean aphid.

Eurasian buckthorns are very difficult to manage once they become established. An assortment of labor intensive management methods have been attempted with varying success. Conventional buckthorn management typically provides only short-term control. Buckthorn easily regenerates from roots or new plants establish from the seed bank. Management of established buckthorn populations is very labor intensive and continual. Buckthorn removal and stump treatment can cost between $1000-$3000 per acre. Due to the high costs and labor-intensive management practices, the vast majority of goes unmanaged.

A long-term solution to buckthorn management could be biological controls, but there has not been sufficient research to determine its potential. Initial research conducted by Center for Applied Bioscience-Switzerland for the Minnesota DNR suggests at least 12 insects that should be evaluated for potential biological control in future research (Gassmann et. al. 2001).

Natural enemies, such as insects, mites and diseases, have an impact on their host plants in their native area. Some of these natural enemies are very host-specific, attacking only one or a few closeley related species. When plants are introduced into new areas, this normally happens without these natural enemies which affect the plants in their area of origin, and as a result the introduced plants are more competitive and may become damagingly invasive, as is the case with *Rhamnus* spp. in North America. Classical biological control of weeds involves the introduction of such host specific natural enemies from the weed's area of origin into areas where the weed has been introduced. Once introduced in the area of invasion, and free from their own predators, these specific natural enemies will exert an increasing stress on the target plants, leading to reduced reproduction, seed dispersal, plant fitness and finally plant populations.

In this case we know that there are specialised natural enemies associated with *Rhamnus* spp. in Europe, and we will test the hypothesis that some of these will prove to be suitable (in terms of host specificity) for introduction into North America, and that ultimately following their introduction they will reduce the competitiveness of *Rhamnus* spp. in North America, so that its adverse effects on human interests are reduced.

The hypothesis is supported by the well documented negative impact of numerous forest pests on a large number of trees and shrubs.

The hypothesis is also supported by successful examples of biological control of trees and shrubs. Although there are relatively few examples of such biological control against woody plants, there is a good track record of success against woody weeds :

Acacia longilifolia : *Trichilogaster acaciaelongifoliae* (Pteromalidae) (Dennill 1988)
Acacia saligna : *Uromycladium tepperianum* (Uredinales) (Morris 1997)
Cordia curassavica : *Metrogaleruca obscura* (Chrysomelidae) ; *Eurytoma attiva* (Hymn., Eurytomidae) (Williams 1951; Simmonds 1980)

Examples of recent biological control programmes against wood invasive plants include :Tamarix *spp. in North America;* and *Ligustrum robustum* spp. *walkeri* (La Réunion Island) (foreign exploration carried out at CABI Bioscience).

Selecting promising biocontrol agents is an inexact science. Criteria proposed for priortizing potential biocontrol agents have little predictive power and produce contradictory rankings (Blossey 1995). Establishment and control success of weed biocontrol agents has been unpredictible (Crawley 1989). Final selection of control agents for buckthorn will be based pre-release studies on host-specificity, effectiveness in reducing plant performance and potential competitive interactions between control agents. Host range testing will follow United States Department of Agriculture's (USDA) process as laid out by the Technical Advisory Group for

Biological Control Agents of Weeds (TAG) (USDA 1998). A feasibility study was carried out in 2001 that list potential control agents for buckthorn and make recommendation for future studies (Gassmann et. al., 2001). The feasibility study also suggests a preliminary plant test list for the host range testing. The ultimate decision on approval for introduction of biological control agents into the United States is USDA-TAG This process may take up to ten years to complete.

Objectives of the research

1. To study the distribution of potential natural enemies of *Rhamnus* spp in Europe, i.e. to locate and further collect potential natural enemies of *Rhamnus cathartica* and *Frangula alnus*. Special attention will be given to a dozen of species which are believed to be specific enough to deserve further according to previous studies and information from the literature. Arthropods of potential interest include include internal root and stem boring species such *Oberea pedemontana* (Col., Cerambycidae), *Synanthedon stomoxiformis* (Lep., Sesiidae) and *Sorhagenia janiszewskae* (Lep., Cosmopterygidae); leaf miners such as *Stigmella catharticella*, (Lep., Nepticulidae), *S. rhamnella*, *Bucculatrix frangullela* (Lep., Bucculatricidae), *B. rhamniella* and *Euspilapteryx quadrisignella* (Lep., Gracillidae); leaf-eating species such as *Sorhagenia lophyllera* (Lep., Cosmopterigidae), *Scotosia vetulata* (Lep., Geometridae) and *Bucculatrix frangullela*(in late larval instar); leaf suckers such as *Heterocordylus erythrophtalmus* (Het., Miridae), and *Psylla rhamnicolla* (Hom., Psyllidae); the leaf gallers *Tetra rhamni* (Acari, Eriophyiidae) and *Trichodermes walkeri* (Hom., Triozidae). The flower feeders *Sorhagenia rhamniella* (Lep., Cosmopterigidae), *Contarinia rhamni* (Dipt., cecidomyiidae) and *Wachtiella krumbholzi* (Dipt., Cecidomyiidae) are not prioritizred in early phase of the project.
2. Host specificity studies on 4-5 species, which have been identified from previous surveys. Insects will be prioritized based on their perceived potential to cause damage to buckthorn by impairing growth and/or reproduction, reduce vigor, or cause structural damage. These factors can potentially lead to buckthorn mortality. Once the highest priority species are selected they will be tested for host specificity.
3. Surveys of buckthorn in Minnesota will be continued to determine what insect species currently utilize buckthorn in Minnesota. Such surveys are needed to determine if any native or non-native insect species are currently found on buckthorn or cause damage to buckthorn.

Methodology

Objective 1. Survey and collect potential natural enemies of *R. cathartica* and *R. frangula*.

Due to the nature of the work, there will be no universal sampling method. Preliminary work will be necessary to gain some knowledge of the distribution of key-insects and their life cycle and the cost of sampling (monetary and time). Therefore, an extensive study is planned and relative estimates will be measured.

Extensive studies : in contrast to intensive studies which involve the repeated observation of the population of a species, extensive studies are carried out over larger areas and are used to provide information on distribution and abundance of species for conservation or management programmes (Morris 1960; Southwood and Henderson 2000). Surveys on *Rhamnus* spp. of varying duration will be carried out in Switzerland, France, Germany, Italy, Yugoslavia, Austria, Poland and other countries if necessary. When possible, a particular area will be sampled twice or at most a few times during the season. The timing of such sampling is obviously of critical importance. It will be variable given the variety of targeted organisms. In addtion to the two targeted plants, other buckthorn species (mainly *Rhamnus alpina*, *R. saxatilis*, *R. pumila*) will be surveyed in order to assess the host range of targeted organisms under natural conditions in Europe.

Relative estimates :
These estimates, in which the number caught cannot be expressed as a density or intensity per area or habitat unit only allow comparisons in space or time (Southwood and Henderson 2000). These estimates are especially useful in extensive work on species distribution such as in this study on the distribution of potential natural enemies on *Rhamnus* spp. in Europe. However, the biological interpretation of relative population estimates is difficult. Size of relative population estimates can be influenced by, e.g. changes in the number of organisms in a particular phase, changes in activity following some change in the environment, changes in the efficiency of the trap or the searching method, and of course changes in actual numbers (Southwood and Henderson 2000).
The most direct approach is the measures of availability : the availability of the population of an animal is the result of the response to the stimuli (efficiency of the searching method), the activity (e.g. due to temperature) and the abundance of the animal. This measure can be defined as the ratio of total catch to total effort. Measures of availability will be used for the immediate assessment of colonization potential of a population and its phenology. They may be used to indicate changes in the diversity of the fauna.

Selecting sampling methods :

Relative methods are particularly appropriate for initial faunal surveys (Southwood and Henderson 2000) :

- Visual inspection followed by capture by hand, or aspirator in fixed-time estimations (catch/unit effort). Again, sampling efficiency will vary with weather, time of the day, period of sampling, etc.
- Beating. In general, beating is only a relative method (catch/unit effort), but with some insects such as lepidopterous larvae or many weevils that fall from the host plant, rather than fly, from the host plant when disturbed, a sufficiently high proportion may be collected for it to be regarded as an absolute method (Southwood and Henderson 2000). The beating method will need to be determined precisely after preliminary sampling. For example, Harris *et al.* (1972) used a 7- by 9-ft white cloth sheet beneath a tree and beat the branches with a 12-ft pole for about 30 seconds. White (1975) used a beating sheet of 36-in^2 and a 3-ft beating stick, and four beats constituted a sampling unit, and a maximum of 10 such units constituted a sample. In a study on the invertebrate fauna on broom, *Cytisus scoparius*, Memmott *et al.* (2000) found that beating collected 87% of invertebrate abundance, 95% of invertebrate biomass, and 100% of phytophagous species found on the branches. A beating tray of 110x86 cm was used and five sharp taps were given to the branches immediately overhanging the sampling tray. As very small insects may be overlooked and active ones may escape, more accurate counts may be obtained by using a screen sieve using which the insects are funnelled into a container (Southwood and Henderson 2000).
- Pheromones. Pheromones lures will be used to catch females of the root-boring Sesiid, *Synanthedon stomoxiformis*. Lures of *Synanthedon myopaeformis* have been effective in

attracting females of *Chamaesphecia hungarica* (Lep., Sesiidae), *Euhagena palariformis* (Lep., Sesiidae) and other sesiid species (I. Tosevski, pers. com., 2002).

Objecitve 2. Host specificity testing

Selection of test plant species

Since the 1970s, the so-called centrifugal phylogenetic method in which plants more- or less-related to the target weed are tested, has become the standard method for determining that host range boundary (Wapshere 1974). Any test plant list usually includes a large number of species closely related to the target species as well as a number of important crop or ornemental species. There is little or no evidence in the existing literature that this approach has ever failed, which suggest that the prediction method is robust (Pemberton 2000). A basic test plant list has been recommended for the biological control of exotic buckthorns in North America (Gassmann et al. 2001). This test plant list includes some 11 species in the genera *Rhamnus* and *Frangula* of which nine are native North American species. It also includes some 13 other species in 10 different genera in the family Rhamnaceae and seven species in two other families in the order Rhamnales. A few other species belonging to other plant orders are also proposed.

Definitions

The target species means the species which is targeted for biological control. The "term target species" is preferred to the term "control species".
The host range is the list of plant species used as hosts. It is necessary to distinguish between the physiological (fundamental) host range and the realized or ecological host range (see below).

No-choice tests: A test in which only one test plant species is available to candidate agents for the duration of the test and therefore include tests with a separate control group on the target species and sometimes a separate no-food control (e.g. in adult no-choice feeding and starvation tests). Note : sometimes it can be useful to test both experienced and naïve candidate agents (i.e.the agent is exposed to the target species prior to the test, or not).

Sequential no-choice tests: A test in which only one test plant species is available to candidate agents for a x-period of time alternatively to the target species for the same x-period of time. Note :The test starts either with the test plant species or with the target species.

Choice tests: Any combination of plant species offered concurrently to candidate agents.

Single choice (paired-choice) test: A combination of one test plant species and one target species offered concurrently to candidate agents

Single choice minus target: A combination of two test plant species offered concurrently to candidate agents, but neither is the target.

Multiple choice test: A combination of more than two plant species offered concurrently to candidate agents in which the target species is always present.

Choice-minus-target: A combination of more than two plant species in which the target species is not present offered concurrently to candidate agents.

The nature of no-choice tests

No-choice larval development or starvation tests measure the ability of a plant to support the development of an immature control agent. No-choice oviposition tests measure the ability of adults to survive and mature, and to lay eggs on a test-plant. Usually small containers or cages are used, but no-choice tests can be conducted in the field by transferring relatively immobile stages onto plants.

It is generally accepted that no-choice tests usually overestimate the true (or realised, or ecological or field) host range of potential control agents (sometimes called false positive results), i.e. the probability of an insect accepting a host in such tests, but then rejecting it in the field are considered high. Indeed, no-choice tests largely ignore the behavioural and ecological constraints that might prevent a control agent using those hosts under natural conditions, but reveal the range of hosts that the agent can use physiologically, in the absence of those constraints (i.e. the physiological or fundamental host range). In summary, no-choice tests give an indication of the fundamental list of plant species on which larvae can complete development or females oviposit. The assumption is that no-choice tests are more conservative with however an important risk of positive result being false and potentially safe and useful agents being rejected. However, it has been argued that, in general, no-choice tests may be more ecologically relevant for inactive, small, passively-dispersed organisms attempting to discriminate between large test-plants. These results also highlight which plants would be at risk if a biological control agent changed its behaviour in the field.
The probability of an insect rejecting a host in such tests, but then accepting it in the field (sometimes called false negative result) is considered very low. To avoid such potentially mis-leading results, special attention must be given the adequate phenology of the plant species, and to the general quality of the plants. Also, oviposition tests are best conducted using mature and experienced insects (insects which have already been in contact with the target species). Sequential no-choice tests have the advantage that the quality and fitness of the female used on the test plant species can be monitored.

The nature of choice tests

There are two types of choice tests. The first type can be subdivided in the single choice (paired-choice) and multiple choice while the second type is represented by the choice test minus target. The implementation of choice tests is usually quite straightforward, the main variables being the number of test plant species included in the test, the size of the testing arena, the stage of the insect used, the number of insects added, the duration of the tests, and the parameters that are scored.
Single choice will be preferred to multiple choice or choice-minus target tests to avoid an hypothetical identification in the hierarchy of adult feeding preferences or oviposition preferences in the presence of more than one plant species and/or in the absence of the target and

its transposition to field conditions. Choice-minus target tests could be carried out only in an attempt to establish a hierarchy of preferences among non target species if necessary or useful. As for sequential no-choice test, sequential “choice with target” and “choice minus target” tests have the advantage to demonstrate the fitness of the potential agent, and, as for multiple choice tests to more rapidly process plant species.

Choice tests are difficult to interpret. They can lead to “false negative” results (e.g. unresponsiveness to lower-ranked species in the presence of higher ranked species), or to “false positive” results (e.g. habituation following repeated contact with non-hosts or volatiles from the target plant permeating the cages and condensing on non-hosts).

A problem common to all types of testing done in cages is the possibility of indiscriminate behaviour, particularly relating to oviposition. Often the problems of cage-induced abberrant behaviour cannot be resolved in the laboratory, and the only solution is to undertake field testing.

Choice versus no-choice tests

Testing of a potential agent control agent usually is the combination of choice and no-choice tests. However, the rationale behind the tests and the order the tests were done varies among authors and or the agent tested. There are almost as many screening procedures as there are agents tested.

One could argue that it would be more logical to focus on tests which follow some of the natural process of host selection (i.e. usually choice tests), but the major problem with choice tests is that acceptable but less preferred host might be protected during tests by the presence of a more highly ranked host. Recent increasing concerns regarding non target impact request that “a plant will not be attacked”. Therefore, priority will be given to no-choice tests in spite of high risks of “false positive” results. Unattacked species will be discarded from further tests unless there is evidence of “false negative” results.

Plants attacked in no-choice tests will be included in increasingly sophisticated tests that expose the control agent to the wider range of cues and barriers insects encounter in the natural process of host selection. In this process, the next step would be to give a choice of plants to a candidate agent in laboratory-based experiments (i.e. paired-choice tests including the target species). Attacked plant species would be then used in confined field-based experiments (e.g. in large “field cages”), and/or in open-field trials.

Testing mature vs immature stages of potential agents

It can be postulated that a particular plant species is safe from attack because it is rejected for oviposition by the females of a potential agent, although its larvae feed on a wide range of plant species. The discrepancy between larval host range and the host range for oviposition by females is likely to occur with several potential agents of buckthorns. When an egg has been laid on a large plant species the neonate larva has no other choice but to feed or to die (this may be untrue for small plant species where larvae have in theory the possibility to move from one plant species to another). Therefore, no-choice larval feeding and development tests will be ecologically appropriate for several of the potential agents of buckthorns. However, oviposition behaviour is the behaviour that will actually determine the realized or field host range of a number of candidate agents. As mentioned above, oviposition tests in a confined environment often lead to either “no eggs were obtained” or “eggs were laid on all plant species and on the cage as well. This is often the case when no particular developments stage of plant or plant parts are needed for the female to oviposit.

Open field tests

Open field host-specificity tests have been suggested as a way of avoiding problems due to containment of the agent and the conservative results they usually generate as the agent is free to express locomotor behaviour associated with host rejection. The use of open field tests has been fairly limited, in part because they were not required in many cases, and in part because of logistic constraints. The majority of open field tests have been carried out to clarifiy apparent anomalies in laboratory-based testing by removing some of the behavioural constraints to host-selection process.

There are two types of open field tests. The interspersion test where potted plant species (paired with potted target plants) are randomly placed in a natural infestation of the target weed. Interspersion experiment relies on natural populations of the agent. The set design experiment combines the target plant and test plant species in a fixed experimental design away from local infestation of the target weed, and deliberate releases of the agent being tested. In general, the results have been fairly clear and have demonstrated a more restricted host range than that shown under confined conditions. However, open-field tests have been criticized on several grounds. The relative abundance of the target and non-target plants in the design may influence behaviour. This has been shown when comparing set design versus interspersion experiments. At normal agent densities, host range is usually more limited in interspersion tests than in set-design tests. Therefore, set design experiments appear less likely to produce false negatives than interspersion experiments in natural infestations of the target weed. The second criticism relates to agent densities which may be much lower in open field tests than potential populations in the country of introduction. This behaviour can be partly assessed in set design experiments by removing the target plants from the experimental plot once the agents are well set up in the experimental plot. The second no-choice phase would reveal the agent's behaviour with regard to non-target plants at high agent densities in the absence of the target species. In conclusion, open-field experiments have an important role in host range testing in some cases but their limits should not be neglected. It should be stressed also that open-field tests are well suited for agents with a relatively low mobility (but mobile enough to get from plant to plant), such as weevil or leaf beetle adults . Very mobile agents may just move away from the experimental plot in particular when target plant populations occur in a relatively close distance. Open-field tests may then be tentatively replaced by large "field cage" tests.

Continuation trials

Often one or more individuals in host range tests oviposit readily or survive much better than the majority of insects tested. Increased survival or oviposition by rare individuals on one plant species may be the means by which evolving populations test host-range boundaries. Such results are difficult to interpret and it is unreasonable to conclude that the plant is not at risk because only a few larvae develop to the adult stage. In the field, populations will be hundreds of times larger than in laboratory. Continuation trials consist of returning adults that have completed development on one plant species during screening tests, onto a fresh plant of the same species in no-choice trials, to test whether the non-target species can support successive generations of the agent. The rationale behind continuation trials is that performance of an agent, especially on a sub-optimal host, may be influenced by the rearing host of its parents. Sometimes, first generation adults reared from a non-target plant failed to produce viable adult progeny, or the decline in total numbers in subsequent generations on a non-target species through reduced performance is possibly a result of trans-generation effects through sub-optimal nutrition. Such studies are rare in biological control. They are difficult to carry out with univoltine species and often only a small number of adults complete development on a non-target plant, such that continuation trials are handicapped when adult emergence is not synchronized. In such cases, length of larval development on a non-target host, pupal weight and adult size may give an indication on the viability and fitness of the subsequent generations produced on the non-target species.

Cut foliage versus whole plants

In host range tests with defoliators in particular, it is common practice to offer the insect portions excised from a growing test plant rather than the whole plant itself. This methodology raises two main concerns: 1) the effect of cutting on plant chemistry or defences, and 2) sampling (i.e. the small portion sampled may omit material of a particular quality that could be utilized by the test insect thereby leading to a false negative result). Interestingly, nine data sets with defoliators showed good agreement between the results of whole plant and cut foliage and there was no clear indication of one method consistently producing high survival. It appears to be no general reason why cut foliage test should not be considered an appropriate method for determining host range, knowing however that some plant groups may be appreciably altered by cutting foliage. Cut foliage will be used for the larval host range of defoliators or free living species. Whole plants will be used for gall-making agents or internal feeders.

Templates for host specificity testing.

A. For potential defoliating lepidoptera species and for each test plant (including target plant) :

- 1 neonate larva of species X each on one buckthorn leaf in a Petri dish; 30 replicates ; leaf change twice a week ; larval development, pupal weight measured ; possibly leaf surface consumed depending on time constraints ; each geographically well separated population will be tested separately (e.g. eastern Austria vs southern Germany ; or Rhine Valley vs Geneva area)

B. For shoot boring moth :

- 1 mated female (or one pair) of species X each on one caged buckthorn or target plant (potted plants) ; 10 replicates ; oviposition, larval development, pupal weight, length of mine assessed.

C. For potential homoptera species :

- oviposition test : 1 mated female (or one pair) of species X each on one caged buckthorn or target plant (potted plants) ; 10 replicates ; # eggs, larval (and gall) development checked twice a week.
- Adult feeding and survival test : 1 adult of species X each on one leaf ; 10 replicates ; survival and longevity are measured ; mean # of punctures if time allows.

Note: The most important plants (or those on which some negative results have been observed) will be tested during two subsequent years to ensure some variability in the insects tested, possibly the plants, and the environmental conditions as well. The number of adults or larvae used in the tests could of course be increased or the design changed.

Analysis: Mean and SE of oviposition rates per plant species, larval stages and survival rate for larval development, mean and SE for pupal weight, mean # days and SE to reach pupal stage, etc will be calculated.

Conclusions

- Lepidoptera and Hemiptera are so far the two main types of potential biological control agents for buckthorns.
- The type of test adopted is related to the type and biology of the agent and how easy it is to rear in lab.
- The number of plant species and the number of replicates that can be offered in any test will depend on the availability of both the test plants and the agents.
- No-choice tests are the basis for assessing the host range of potential biological control agents for exotic buckthorns in North America.

Lepidoptera – external feeders (defoliators)

- No-choice larval feeding and development tests are the basis for assessing the host range of Lepidoptera with externally feeding larvae.
- Length of larval development, pupal weight and adult size reared from various plant species will be compared.
- Those plant species accepted for larval development will be used in single-choice laboratory-based or single-choice field-cage based oviposition tests.
- Depending on the results of the single-choice oviposition tests, a two-phase set design field experiment will be carried out with plant species accepted for larval development in no-choice tests.

Hemiptera

- No-choice larval development and no-choice adult feeding and oviposition tests are the basis for assessing for assessing the host range of Hemiptera. No-choice feeding tests of Hemiptera adults may not be necessary where it is the larvae that are most damaging.
- Length of larval development, pupal weight and adult size reared from various plant species will be compared. Adult longevity and fitness will also be compared.
- Those plant species accepted for oviposition and/or larval development will be used in single-choice laboratory-based or in single-choice field-cage based adult feeding and oviposition tests.
- Depending on the results of the single-choice tests, a two-phase set design field experiment will be carried out with plant species accepted for adult feeding and oviposition and/or larval development in no-choice tests.

<u>Internal feeders – gall makers</u>

- The host range of internal feeders or gall makers will be based on no-choice oviposition and larval development laboratory-based tests.
- Those plant species accepted for oviposition and larval development will be used in single-choice laboratory-based or single-choice field cage based tests.
- If no oviposition is obtained in captivity, larval transfers will be made to assess the larval host range of those agents (i.e. no-choice larval development tests).
- Depending on the results of the single-choice tests and/or the no-choice larval development tests following larval transfer, a two-phase set design field experiment will be carried out with plant species accepted for oviposition and/or larval development in no-choice tests.
- Both cut foliage and whole plants will be used depending on the type of agent.

- The natural field host range will be assessed by comparing the insect complex associated on a number of *Rhamnus* species and on literature records

Objecitve 3. Survey of insect on buckthorn in Minnesota

Sites surveyed: Seven sites were identified for repeated sampling in southern Minnesota with four representing urban landscapes and three in rural landscapes. The four urban landscapes include: Battle Creek Park in St. Paul (Ramsey County), University of Minnesota St. Paul Campus (Ramsey County), and Tierney Wood's and Hyland Park in the Three Rivers Park District (Hennepin County). The three rural landscapes are located south of the Twin Cities metro area, including: UMORE Park, University of Minnesota Outreach, Research- Experiment station in Rosemont (Dakota County), Frontenac State Park (Goodhue County), and Courthouse Park in Waseca (Waseca County). Two of these sites are located along the Mississippi River bluffs (Battle Creek Park and Frontenac State Park) while the remaining sites are more upland locations.

Sampling Techniques: Each site will be surveyed twice monthly. At each site, twelve trees will be marked and tagged. Of the twelve designated trees, there will be four small, four medium, and four large trees. On the medium and small trees all branches are checked for insects or insect damage. Only reachable branches from the ground were checked on the large trees. Any insects or eggs that are found will be removed, returned to the laboratory and placed into Petri dishes to allow immatures to develop to the adult stage. Records will be maintained on each insect as the site, date collected and which individual tree it was collected from. As adults emerged insects will be dispatched, and pinned for later identification.

To insure we are observing as many insects feeding on buckthorn as possible, additional sampling along two transects will be conducted at each site monthly. The first transect will occur along a path, roadway or other opening where buckthorn has full exposure to the sun. A second transect, perpendicular to the first transect into the center of a buckthorn infestation will be surveyed. The second transect often includes plants growing in the understory where seedlings are in shade or filtered sunlight throughout the day. Along each transect, 25 consecutive buckthorn trees will be visually surveyed for insect presence. Any insects or eggs that are found will be removed, returned to the laboratory and placed into Petri dishes to allow immatures to develop to the adult stage. Records will be maintained on each insect as the site, date collected and which individual tree it was collected from. As adults emerged insects will be dispatched, and pinned for later identification. We anticipate making comparisons among sites as to species collected in sunny open locations vs. shaded locations. If differences are found we will increase the number of transects next year to allow comparison among transects within a single site.

Description of Results

From this research, we expect to expand exporatory efforts to indentify potential control agents in Europe. In addition, the first two years of host specificity testing will be complete for the top 3-5 control candidates. Products will include a list of potential control agents discovered, summary of host specificity testing for control agents tested and finally a list of recommendations for further research efforts.

Project Timetable

Project Milestones	Date
Write contract University of MN	7/2005
Carry out field surveys for insect on buckthorn in MN	9/2005
Write contract with Center for Applied Bioscience- Switzerland	12/2005
First semi-annual status report	1/2006
Continue host specificity testing of potential agents in Europe	4/2006
Continue field surveys for potential agents in Europe	5/2006
Second semi-annual status report	8/2006
Third semi-annual status report	2/2007
Continue screening of potential agents	4/2007
Fourth semi-annual status report	8/2007
Evaluate the extent of future work effort needed on potential insects	1/2008
Fifth semi-annual status report	3/2008
Final Report with all findings and recommendations	6/2008

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