Result 2: Survey of insects on buckthorn in Minnesota

Common buckthorn, *Rhamnus cathartica* L.: Available feeding niches and the importance of controlling this invasive woody perennial in North America

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Summary

Common buckthorn, *Rhamnus cathartica* L., an invasive woody perennial of northern hardwood forests in North America, has been targeted for classical biological control and research has been underway since 2001. In support of biological control research, a survey was conducted for insects associated with common buckthorn in a portion of its introduced range in the state of Minnesota. This survey provides baseline information on available feeding niches for potential control agents of common buckthorn and identifies the natural enemy community that could potentially interfere with agent establishment. In two years of sampling, 356 species representing 111 families and 13 orders were collected from common buckthorn in Minnesota. There was no significant defoliation observed at any of the study sites. We surmise that ample feeding niches are available given that most herbivores collected can be classified as generalists. However, the abundance of parasitoids and predators may hinder establishment of potential biological control agents. Further research is needed to determine if biotic resistance could play a significant role in preventing establishment of herbivores in a classical biological control programme for common buckthorn in North America.

Keywords: Rhamnaceae, arthropod herbivores, natural enemies, biological control

Introduction

Common buckthorn, *Rhamnus cathartica* L., is an invasive woody perennial that has become established in northern hardwood forests of North America. It was introduced as a landscape plant and used as a shelterbelt tree because of its winter hardiness and its ability to grow in multiple soil types and habitats (Archibold et al., 1997). In North America, common buckthorn is one of the most invasive woody perennials in natural ecosystems (Archibold et al., 1997; Catling, 1997). Common buckthorn retains its leaves longer than native tree species, creating a competitive advantage (Harrington et al., 1989). In addition, Archibold et al. (1997) suggested that common buckthorn might be allelopathic, allowing its seedlings to grow below mature female trees while inhibiting native tree species. Common buckthorn produces a dense branching structure that attracts nesting songbirds; however, the American robin, *Turdus*

migratorius L., experiences higher levels of predation when nesting in common buckthorn compared to native species (Schmidt and Whelan, 1999). Others have documented that common buckthorn causes changes in soil properties, leaf litter composition, and micro-arthropod communities (Heneghan et al., 2002; 2004).

Common buckthorn has negative impacts on agriculture. It is the spring host for oat crown rust, *Puccinia coronata* Corda, which can cause severe yield losses in oats (Harder and Chong 1983). Common buckthorn was identified as a suitable overwintering host for soybean aphid, *Aphis glycines* Matsumura, which was first discovered in North America in 2000 and by 2007 has spread to 24 states and 3 Canadian provinces (Ragsdale et al., 2004; Voegtlin et al, 2005).

Common buckthorn is currently on the noxious weed list in six states and two Canadian provinces (University of Montana-Missoula, 2007; USDA, 2007). Multiple methods of control have been employed against common buckthorn including cut stump treatments, foliar herbicide applications, and burning (Archibold et al., 1997). Such control efforts are expensive and for the most part are only effective on a small scale because seedlings tend to re-grow after a burn or a chemical treatment (Archibold et al., 1997). In the early 1960s, several potential biological control agents were identified by Malicky et al. (1970), but their study was not continued. In 2001, biological control research was resumed by the Minnesota Department of Natural Resources in collaboration with CABI Europe Switzerland (CABI) to identify and screen potential control agents.

Our first objective was therefore to conduct a survey of herbivorous insect species associated with common buckthorn, while our second objective was to identify which predators and parasitoids are found on common buckthorn in Minnesota. These data will provide key information in understanding the availability of feeding niches for potential biological control agents and provide insights on what biotic resistance might be present to interfere with agent establishment in Minnesota.

Methods and Materials

Field sites

In 2004 and 2005, eight common buckthorn sites were sampled in three different habitat types, i.e. urban (three sites), rural (two sites), and agricultural (three sites), in seven (2004) or six (2005) southern Minnesota counties (see Yoder 2007 for specific locations). Sites were characterized for their plant communities by randomly sampling ten, $1m^2$ plots. Data collected in each plot included: percent cover of common buckthorn, percent cover of other plant species, common buckthorn stem density, other plant species stem density, number of different plant species, and percent canopy cover. Canopy cover was estimated using a densiometer. To characterize mature trees in the forest, which were not captured by the $1m^2$ plots, we counted the number of trees for each species that were at least 1.5m in height, in a 2m radius around each $1m^2$ plot.

Insect sampling

In 2004 and 2005, 12 common buckthorn plants: four small (<1m in height), four medium (1-3m), and four large (>3m), were marked for repeated insect sampling at each site. Sites were visited every two weeks throughout the growing season (15 June - 15 September 2004; 15 May - 15 September in 2005). All reachable branches were visually surveyed and any insect present was collected, and immediately returned to the laboratory for either identification

if adults were captured or reared to adult stage if immature insects were collected. In addition to the 12 plants sampled biweekly, two transects were established at either six (2004) or five (2005) of the largest sites. The first transect consisted of 25 consecutive common buckthorn trees growing along a path, roadway, or other opening where trees had full exposure to the sun resulting in common buckthorn trees that were larger. The second transect was perpendicular to the first transect and consisted of another 25 consecutive common buckthorn trees and included trees growing in the under-story in shade or filtered sunlight. All trees selected were visually surveyed for up to 2 minutes and all insects observed were collected and returned to the laboratory.

Adults reared and collected in the field were preserved and pinned for later identification. Soft-bodied insects and immature insects that failed to reach the adult stage were preserved in vials containing 70% ethanol. Voucher specimens were deposited in the Entomology Museum at the University of Minnesota.

All adult specimens were categorized as herbivores, predators, parasitoids, or scavengers. For a species to be included in the statistical analysis, a minimum of five specimens per species was required. A qualitative Sorenson index (Magurran, 1988) was used to characterize differences in insect assemblages between habitat types. The equation for the qualitative Sorenson index (C_S) is $C_S=2j/(a+b)$ where j is the number of species found in both groups, a is the number of species in group x, and b is the number of species in group y. We used a quantitative Sorenson index (Magurran, 1988) to characterize differences in insect assemblages in relation to abiotic factors such as the amount of sunlight (forest edge vs. interior) and biotic factors such as tree size (small, medium, large). The equation for the quantitative Sorenson index (C_N) is $C_N=2_{jN}/(aN+bN)$ where jN is the sum of the lower of the two abundances recorded for a given species found in both groups, aN is the total number of specimens in group x, and bN is the total number of specimens in group x, and bN is the total number of specimens in group x, and bN is the total number of specimens in group x, and bN is the total number of specimens in group x.

Results

Site characteristics

Overall, urban sites had the highest density of common buckthorn and the lowest plant species diversity (Table 1). Those sites characterized as agricultural sites had the opposite, with the lowest density of common buckthorn and the greatest plant diversity (Table 1). Those sites characterized as rural had an intermediate percent cover of common buckthorn, but on a stem density per m² had common buckthorn densities equal to those of the urban landscapes. Plant species diversity was low in the rural sites, but the percent cover of other plant species and stem density of other plant species was intermediate (Table 1). Interestingly, if common buckthorn was excluded from this analysis, the most common plant species found in either urban or rural sites was garlic mustard, Alliaria petriolata L., another invasive plant of hardwood forests. When surveying the mature tree composition, four sites had common buckthorn as the dominant mature tree. Urban sites had a significantly higher density of mature common buckthorn trees when compared to rural or agricultural sites with one urban location having a maximum of 11 mature buckthorn trees per $1m^2$. Agricultural sites had the lowest number of mature buckthorn trees and the highest number of other mature tree species (Table 1). For all sites, the most dominate mature tree species, other than common buckthorn, was American elm, Ulmus americana L., followed by box elder, Acer negundo L.

Insect fauna

Over the two-year study, a total of 1,733 arthropods representing 13 orders, 111 families and 356 species were collected from common buckthorn. Hemiptera was the most abundant order, followed by Hymenoptera, which consisted mostly of parasitoids (Tables 2 & 3). Several species were abundant, each with over 75 specimens collected: *Metcalfa pruinosa* (Say) (Flatidae), *Lasius alienus* (Förster) (Formicidae), *Harmonia axyridis* (Pallas) (Coccinellidae), *Graphocephala coccinea* (Forster) (Cicadellidae), and *Trissolcus* sp. a. (Scelionidae).

For the analysis we used 606 herbivores representing 32 different species, 154 predators representing 5 different species, and 140 parasitoids representing 4 different species (Table 2 & 3). An additional 314 species were excluded from analysis because fewer than five specimens were collected over the 2 year sampling effort or because species were known to be saprophagous, mycetophagous, scavengers, or non-feeding as adults. The Sorenson index (C_S) showed that all three habitat types, agriculture, rural, and urban landscapes, were very similar in insect species diversity (range 0.71-0.73). The majority of predators were captured at sites in agriculture habitats (56%) and the majority of parasitoids were captured in rural habitats (61%). The quantitative Sorenson index for insect diversity for forest edge and interior using data collected from the two perpendicular transects was ($C_N=0.54$). It is not surprising that transects are different since more insects were collected on common buckthorn along the transects where plants were along a forest edge (62% of captures) compared to the interior (38% of captures). When comparing tree sizes, large and small trees had the least similar insect composition ($C_N=0.59$). Medium trees tended to have higher diversity and abundance compared to large and small trees.

In general, there was very little evidence of feeding damage on common buckthorn. The most common type of damage was leaf miner tunnels, followed by damage caused by lepidopteran larvae. Nine species were reared in the laboratory after collecting immature insects from common buckthorn indicating these nine species are able to complete their development solely on buckthorn. These included three hemipteran species, *Acanalonia conica* (Say), *M. pruinosa*, and *Gyponana quebecensis* (Provancher), three orthopterans, *Neoxabea bipunctata* (De Geer), *Oecanthus fultoni* Walker, and *Oecanthus niveus* (De Geer), and three lepidopterans collected as eggs and reared to adult, which included *Choristoneura rosaceana* (Harris), *Machimia tentoriferella* Clemens, and *Spilosoma virginica* (Fabricius). The two tortricids, *C. rosaceana* and *M. tentoriferalla* experienced high mortality during rearing and adult specimens that did emerge often had abnormal wing development. However, a literature search revealed that these nine species listed above can be categorized as generalist herbivores and are not considered specialist herbivores that only feed on common buckthorn.

Discussion

Urban sites, which had the densest common buckthorn infestation and lowest plant species diversity, also had the lowest insect abundance when compared to other habitat types. All urban sites sampled were located in highly populated areas where human activities could easily disturb the natural habitat. In contrast, agricultural sites had the highest plant diversity with more insects collected at those sites. Predators were collected at higher rates in agricultural sites than the other sites possibly drawn there by agricultural pests that would be found in the adjacent crop fields.

The main objective of this study was to identify major herbivores present on common buckthorn in Minnesota. Overall, there were many herbivores collected, however, most insects collected were represented by fewer than five specimens suggesting that they were transient

feeders or generalist herbivores that do not utilize common buckthorn. In reports of herbivores collected from *R. cathartica* in Europe, the most common insect species found were Lepidopterans (Malicky et al. 1970). Here we show that in Minnesota, defoliators were common, but unlike Europe more Hemipterans were encountered in Minnesota than Lepidopterans. During our 2-year study we did not find any insect feeding internally on buckthorn, and thus one potential niche that could be exploited successfully would be an internal feeder such as the stem-boring beetle, *Oberea pedemontana* Chevrolat (Coleoptera: Cerambycidae) which has been identified in Europe as a possible biological control agent of *R. cathartica* (Gassmann, 2005). Even though we found many generalist herbivores feeding on leaves at no time did defoliation exceed 5% on any one tree, thus a specialist herbivore would have an abundant resource to utilize in Minnesota.

The second objective of this study was to identify possible sources of biotic resistance if non-native herbivores were introduced as classical biological control for common buckthorn. There were numerous parasitoids and predators, all considered generalists, collected from common buckthorn. The abundance of parasitoids and predators may indeed hinder establishment of potential biological control agents. Generalist predators have been known to interfere with biological control agents released for purple loosestrife control (Sebolt and Landis 2004). Currently, there have been a few species proposed as potential biological control agents for common buckthorn in North America (Gassmann 2005). As agent selection continues for common buckthorn, the species diversity and abundance of natural enemies collected from buckthorn documented here needs to be considered. In particular H. axyridis, could play a significant role in preventing establishment of herbivores since it was the most abundant generalist predator collected and this coccinellid is known to prefer arboreal habitats. This recently introduced coccinellid was more common in spring and fall (Figure 1). Harmonia axyridis could exert strong biotic resistance on biological control agents especially if a vulnerable life stage was present when H. axyridis densities were high. For example, one group of candidate biological control agents for common buckthorn are the psyllids, Cacopsylla rhamnicolla and Trichohermes walkeri (Gassmann 2005) and it is possible that Harmonia axyridis would pose a particular threat to psyllids. This potential negative interaction could be studied as part of the host testing procedure.

Acknowledgements

We would like to thank Dr. John Luhman, Dr. Leonard Ferrington, and Gregory Setliff for help on identifications. In addition, we would like to thank all of the undergraduate researchers for help in the field and laboratory. This research was funded by the Minnesota Department of Natural Resources based on funds appropriated by the Minnesota Legislature as recommended by the Legislative Commission on Minnesota Resources.

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Table 1. Site characteristics for three habitat types (urban, rural, and agricultural) surveyed for insect fauna on *Rhamnus cathartica*, common buckthorn.

All vegetation (1 m ²)	Urban sites	Rural sites	Agricultural sites
% Cover of common buckthorn	61.0 ± 0.1	48.0 ± 0.1	31.0 ± 0.1
% Cover of other plant species	39.0 ± 0.1	52.0 ± 0.1	69.0 ± 0.1
Common buckthorn stem density m ²	6.1 ± 1.1	6.1 ± 1.1	4.3 ± 0.9
Other plant species stem density m ²	11.5 ± 2.4	28.4 ± 3.4	26.4 ± 3.6
Number of other plant species	3.5 ± 0.4	3.8 ± 0.3	6.8 ± 0.5
% Canopy cover	82.0 ± 1.1	83.0 ± 0.8	80.0 ± 0.8

Mature Tree Survey (12.56 m²)^a

Number of common buckthorn trees	6.1 ± 1.5	2.8 ± 0.7	1.1 ± 0.3
Number of other trees	1.9 ± 0.4	1.4 ± 0.2	2.6 ± 0.3
Number of other tree species	1.5 ± 0.3	1.1 ± 0.1	1.4 ± 0.1

^a, trees at least 1.5 m tall in a 2 m radius from center of plot (12.56 m²)

Table 2. Herbivores collected on *Rhamnus cathartica*, common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included, except for *Oecanthus* spp., because of the high abundance of immature specimens collected.

Order	Family	Genus species	2004	2005	Total
Orthoptera	Gryllidae	Neoxabea bipunctata (De Geer)	10	5	15
		Oecanthus fultoni Walker	2	1	3
		Oecanthus niveus (De Geer)	1	2	3
Subtotal			13	8	21
Hemiptera	Acanaloniidae	Acanalonia conica (Say)	9	11	20
	Aphididae	Aphis glycines / nasturtii	0	39	39
		Aphis glycines Matsumura	7	17	24
		Aphis nasturtii Kaltenbach	0	26	26
	Cercopidae	Clastoptera obtusa (Say)	0	7	7
		Philaenus spumarius (L.)	2	8.	10
	Cicadellidae	<i>Empoasca sp</i> . b	0	5	5
		Graphocephala coccinea (Forster)	21	64	85
		Gyponana quebecensis (Provancher)	4	9	13
		Jikradia olitorius (Say)	9	6	15
	Derbidae	Cedusa incisa (Metcalf)	8	2	10
	Flatidae	Metcalfa pruinosa (Say)	11	164	175
	Miridae	Hyaliodes harti Knight	7	6	13
		Hyaliodes vitripennis (Say)	3	2	5
		Paraproba capitata (Van Duzee)	6	8	14
		Phytocoris spicatus Knight	2	4	6
	Pentatomidae	Euschistus tristigmus (Say)	8	9	17
	Tingidae	Corythucha pergandei Heidemann	0	5	5
Subtotal			97	392	489
Coleoptera	Chrysomelidae	Diabrotica longicornis (Say)	1	5	6

No. of specimens

	Curculionidae	Polydrusus sericeus (Schaller)	2	5	7
	Pyrochroidae	Pedilus impressus (Say)	1	4	5
Subtotal			4	14	18
Lepidoptera	Arctiidae	Spilosoma virginica (Fabricius)	0	10	10
	Gracillariidae	Phyllonorycter caryaealbella (Chambers)	2	6	8
	Psychidae	Thyridopteryx ephemeraeformis (Haworth)	0	5	5
	Tortricidae	Choristoneura rosaceana (Harris)	4	5	9
		Machimia tentoriferella Clemens	2	3	5
Subtotal			8	29	37
Diptera	Cecidomyiidae	Parwinnertzia notmani Felt	2	5	7
Hymenoptera	Cynipidae	Diplopepsis sp. a	0	9	9
		Liodora sp.	0	6	6
	Tenthredinidae	Fenusa sp.	0	19	19
Subtotal			0	34	34
Totals			122	477	599

Table 3. Predators and parasitoids collected on *Rhamnus cathartica*, common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included.

Order	Family	Genus species	2004	2005	Total
Hemiptera	Nabidae	Lasiomerus annulatus (Reuter)	10	15	25
Coleoptera	Cantharidae	Podabrus rugulosus LeConte	5	0	5
	Coccinellidae	Coleomegilla maculata DeGeer	3	2	5
		Harmonia axyridis (Pallas)	44	68	112
			52	70	122
Diptera	Empididae	<i>Tachypeza</i> sp. a	4	3	7
Hymenoptera	Platygasteridae	Leptacis sp. c	0	8	8
	Scelionidae	<i>Idris</i> sp.	18	0	18
		Trissolcus sp. a	37	39	76
		Trissolcus sp. b	0	38	38

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No. of specimens



Figure 1. Seasonal abundance of *Harmonia axyridis* observed on *Rhamnus cathartica*, common buckthorn in Minnesota. Data pooled for 2004 and 2005.