

THESIS

CONTROL AND DISPERSAL OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA L.*)

Submitted by

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ABSTRACT

CONTROL AND DISPERSAL OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA L.*)

Russian olive (*Elaeagnus angustifolia L.*) is a noxious, perennial tree or shrub that has invaded thousands of acres across the western United States. Trees are saline tolerant, drought tolerant, form actinorhizal associations with *Frankia* spp. to fix nitrogen and are actively dispersed by birds. Two areas that were researched for this thesis included the control of Russian olive trees through applications of aminocyclopyrachlor and the second being dispersal of Russian olive seeds by European Starlings (*Sturnus vulgaris*).

Aminocyclopyrachlor is in a new family of chemistry called the pyrimidine carboxylic acids under the synthetic-auxic herbicides mode of action. We examined several different application techniques (e.g. cut stump, basal bark and hack and squirt applications) to assess the efficacy of aminocyclopyrachlor. The majority of our work consisted of cut stump applications, where we performed two studies evaluating the season of application of aminocyclopyrachlor to Russian olive trees, the effect of size of Russian olive trees on efficacy of aminocyclopyrachlor. Whether cut stump application occurred in summer, fall, or winter, herbicides controlled Russian olive similarly, but not all trees were killed with a single treatment across all timings. Aminocyclopyrachlor at 1 and 2.5% applied in summer controlled 100% of Russian olive. A fall application of 5% aminocyclopyrachlor and a winter application of 30% triclopyr ester similarly killed all

trees. Land managers targeting woody species consider 100% control to be acceptable and any regrowth requiring re-treatment is not acceptable. We examined “regrowth factor” as a means to quantify success taking into account the number and height of shoots 1 YAT at one field site. Regrowth was highest in the no herbicide/no oil check population, followed by the 100% Bark Oil Blue LT, 1 and 2.5% aminocyclopyrachlor in fall and 5% aminocyclopyrachlor in both fall and winter. No regrowth was recorded for the triclopyr ester for all three application timings as well as the summer and winter applications of 1 and 2.5% aminocyclopyrachlor, and the fall application of 5% aminocyclopyrachlor. Tree size is an important factor influencing efficacy and the interaction between treatment and site was significant where herbicides were more effective on smaller trees. For 3 to 9 inch trees, herbicide treatments controlled Russian olive similarly (88-100% control) and more effectively than the 100% JLB oil PLUS (50%) and the no herbicide/no oil check trees (47%). No herbicide treatment killed all 9 to 15 inch Russian olive trees 1 year after treatment (1YAT), but 1% and 2.5% aminocyclopyrachlor with JLB oil PLUS, 30% triclopyr ester with JLB and 2.5% aminocyclopyrachlor with Bark Oil Blue LT controlled 88 to 93% of trees in this size class. Regrowth from larger trees was observed to be highly variable within treatments.

We also examined the efficacy of aminocyclopyrachlor for the hack and squirt methodology as a means to control Russian olive trees. We compared hack and squirt applications of aminocyclopyrachlor to imazapyr (Habitat), glyphosate (Rodeo), aminopyralid (Milestone), triclopyr amine (Garlon 3A), Milestone VM+ (10:1 triclopyr amine + aminopyralid), and a 50:50 aminocyclopyrachlor + triclopyr amine mixture. Trees were ‘hacked’ using a hand held ax for every 3 inches of trunk diameter and then

injected with 1 ml of undiluted herbicides. We found that aminocyclopyrachlor controlled 91% of Russian olive trees 1YAT. This level of control was comparable to both industry standards, glyphosate and imazapyr (94% and 98%, respectively). A 50:50 mixture of aminocyclopyrachlor and triclopyr amine controlled 98% of Russian olive trees 1YAT. Products containing aminopyralid (e.g. Milestone and Milestone VM+) had lower mean control (84% and 89%, respectively), than aminocyclopyrachlor. Triclopyr amine had the lowest percent control when applied alone at both field sites (77%). From our studies we showed that aminocyclopyrachlor is comparable to industry herbicide standards and thus, is an effective tool to control Russian olive trees by cut stump and hack and squirt applications.

Previous research identified 51 different species of birds that fed on and likely dispersed Russian olive seeds. European Starlings are invasive birds that were introduced into North America in the 1890s. We used field photographic observations and a controlled feeding study to gain a better understanding of the role European Starlings play in dispersal of Russian olive seeds. Our data indicate that indeed this bird species is a potential dispersal of Russian olive. Two studies were conducted to examine the dispersal of Russian olive seeds by European Starlings. In the first study, Russian olive trees were monitored for 1 year at two field sites to determine feeding behaviors of wild animals on Russian olive seeds using two trail cameras (WSCA01 Wing-Scapes Birdcam and a Moultrie MFH-DGS-I60 Game Spy digital camera). In the second study, 20 European Starlings were collected in the field and housed at the USDA-NWRC research facility in Ft. Collins, CO. Birds were kept in individual cages during the experiments where they were fed 25 Russian olive seeds per day and monitored for

behavior using a 17 hour camcorder. Germination and viability of ingested seeds were compared to control seeds, hulled seeds, seeds ground on sandpaper and nicked, and seeds soaked in 17.8 M sulfuric acid (H_2SO_4) for 1hour. In the observational study, we determined that European Starlings did feed on Russian olive seeds in the wild, particularly in the late fall and early winter months (November to December). We also learned from the controlled experiment that European Starlings readily consume Russian olive seeds, with the majority of seeds being regurgitated after a 30 minutes of digestion. Digested seeds had the highest level of germination (57%) compared to hulled seeds (40%) and ground/nicked seeds (30%). Viability tests confirmed that digested/regurgitated seeds remained viable (87%) and had no net loss in viability after consumption.

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Chapter 1: Thesis introduction

THESIS INTRODUCTION

Since its introduction into the United States in the early 1900s Russian olive (*Elaeagnus angustifolia L.*) has become the fifth most dominant woody, riparian species in the western United States (Friedman et al. 2005, Zouhar 2005). Russian olive is currently found throughout most of the country, except 13 states in the southeast (Katz and Shafroth 2003). Much of the debate over the invasiveness of Russian olive stems from the western states where trees have been observed to be invasive since 1924 (Christensen 1963, Brock 1998, Stannard et al. 2002). Primarily, Russian olive is considered invasive in many riparian habitats where it can alter the ecosystem dynamics such as vegetation structure (both composition and canopy), nutrient cycling, hydrology, succession, and wildlife abundance and diversity (Tu 2003).

Plant Characteristics

Russian olive is a member of the Elaeagnaceae family, and closely related to silverberry (*Elaeagnus commutate* Bernh. Ex Rydb), buffalo berry (*Shepherdia Canadensis*(L.) Nutt.) and autumn olive (*Elaeagnus umbellate* Thunb) (Katz and Shafroth 2003). Russian olive is a perennial, deciduous tree or a multi-stemmed shrub, typically with reddish bark that can sometimes be shredded in appearance (Katz and Shafroth 2003). Leaves are silvery grey-green, lanceolate-shaped and alternate along the branches (Zouhar 2005, Brock 1998). Branches are often armed with sharp, 2 to 3 cm long thorns (Zouhar 2005). Trees range between 8 and 40 feet tall, but are capable of growing 45 feet tall, with trunks ranging between 4 and 20 inches in diameter (Stannard

et al. 2002, Zouhar 2005). When Russian olive trees are permitted to grow in close proximity to one another they can form dense groves or thickets (Stannard et al. 2002).

Russian olive trees often flower and set seed within 3 years of establishment (Tu 2003). Large numbers of small, aromatic yellow flowers are produced in early spring that are pollinated by insects (Katz and Shafroth 2003, Zouhar 2005, Olson and Barbour, 2002). In late spring trees produce a bountiful crop of silvery to yellow drupe-like fruits with a hard-coated seed surrounded by a fleshy perianth (Olson and Barbour 2002, Stannard et al. 2002). Fruits are highly attractive to birds who readily feed upon the nutrient rich pulp by ingesting the whole fruit (Jinks and Ciccarese 1997, Zouhar 2005). It is hypothesized that seeds are ingested with the fruit, then pass through the digestive tract of the birds, and are deposited in new areas where they can quickly spread (Olson and Knopf 1986). Seeds remain viable for up to 3 years under normal storage conditions (Stannard et al. 2002).

Previous research indicated that Russian olive seeds have an average viability of approximately 86% (Olson and Barbour 2002); however, this viability is mediated by several factors. Russian olive seeds require a period of afterripening to germinate (Katz and Shafroth 2003). Afterripening can occur in two ways; 1) a period of chilling, or 2) scarification of the seed coat. A period of roughly 10 to 90 days between 1 and 10 C, with colder temperatures leading to shorter time periods, is needed for cold stratification of Russian olive seeds to break dormancy (Olson and Barbour 2002). Naturally, seeds require an afterripening period of 2 to 3 months around 5 C (41 F) to break dormancy (Zouhar 2005). Russian olive seeds are considered “hard seeded” and may require scarification with either sulfuric or hydrochloric acid for ½ to 1 hour (Olson and Barbour

2002, Shafroth et al. 1995). A non-leachable germination inhibitor has been identified in the seed coat, endocarp and the fleshy perianth of Russian olive seeds (Hamilton and Carpenter 1975, Hogue and Lacroix 1970, Jinks and Ciccarese 1997). Removal of the seed from the exocarp can increase seed germination by 50 to 60% (Jinks and Ciccarese, 1997, Hamilton and Carpenter, 1975). Hogue and LaCroix (1970) performed germination trials on whole seeds, seeds that had been excised from the outer fruit, and embryos. Seeds were plated in petri-dishes and covered with water soaked filter paper for several days. Hogue and LaCroix found that whole seeds had 0% germination over the testing period (7 days), whereas excised seeds had 58% after 7 days, and embryos had 98% germination after 2 days. In the same paper, Hogue and LaCroix performed a second study on seeds where the endocarp and seed coat had been removed exposing the embryo. Two-hundred whole Russian olive seeds were soaked in a solution of “leachate” that was meant to leach out any of the germination inhibitors in the seed coat. After 2 weeks of soaking none of the seeds germinated. When a small sample of the “leachate” was applied to lettuce seeds they found a strong germination inhibition (2% germination for “leachate” applied, and 6% for a non-treated control). Hogue and LaCroix concluded that this germination inhibitor had a strong positive affect in controlling the germination of Russian olive seeds. Hamilton and Carpenter (1975) examined this germination inhibitor and found that it was in the highest concentration in the outer seed covering. Later analyses of the germination inhibitor were conducted to discover the identity of this substance. Samples were compared using an absorbance spectrum and compared to the known germination inhibitors absisic acid and coumarin. From the absorbance spectrum

calculations Hamilton and Carpenter determined that the unknown substance (peak at 271nm) was more similar to coumarin (peak: 275nm) than to abscisic acid (peak: 265).

Below ground, Russian olive trees produce vast root systems, often supported by creeping lateral root runners, which can associate with the nitrogen fixing bacteria *Frankia* sp., forming an actinorrhizal association (Zouhar 2005). A few reports in the literature indicate Russian olive can form associations with mycorrhizal fungi, especially in its native range. In a survey of 1,280 1 cm root segments harvested from Russian olive trees in North Dakota nurseries, Riffle (1977) found that 77% of his samples either had established or developing vesicles or arbuscules from mycorrhizal fungi.

Russian olive trees are reported to be tolerant of many different adverse soil conditions, such as salinity or drought (Stannard et al. 2002, Zouhar 2005). Trees are tolerant of up to 10-15 g/L soil salinity, with a typical average range between 0.1 to 3.5 g/L (Zouhar 2005). Russian olive trees are fairly drought tolerant and may grow in upland areas with as little as 8 to 10 inches of mean annual precipitation (Stannard et al. 2002). Russian olive trees may reproduce asexually from lateral roots, in particular, following management applications that do not result in complete death of the trees (Caplan 2002). In addition, Russian olive may successfully regenerate asexually when it is close to water (Pearce and Smith 2001).

Russian olive trees exhibit a wide range of growth forms and patterns that typically are site dependent and vary based upon the surrounding plant community composition, structure, and disturbance regime (Zouhar 2005). Russian olive trees are usually found on sites with moderate disturbance, but are often termed a late seral species. Russian olive trees can establish early in succession due to their tolerance of

nutrient deprived poor soils and nitrogen fixation associations with *Frankia* spp., and positive mycorrhizal associations (Zouhar 2005). Owing to nitrogen fixation, Russian olive leaves have high nitrogen content and may increase soil available nitrogen from leaf litter decomposition, thus further changing succession (Zouhar 2005).

While tolerating many environments Russian olive trees have specific elevation and temperature ranges in which they are most abundant. Friedman et al. (2005) examined 500 USGS river gauging stations over the western United States. At each station visual observations were made on the abundance of vegetation. From the survey it was determined that Russian olive is uncommon above elevations of 2,296 m (Friedman et al. 2005). Friedman (2005) found that Russian olive was not present at 40 of 500 sites which had mean annual minimum temperatures of less than -8.7 C; meaning that sites above this threshold would not be conducive for Russian olive establishment.

History

Russian olive is native to Eurasia and southern Europe (Katz and Shafroth 2003). In its natural range Russian olive occurs in small clumps and is primarily found in coastal areas, riparian corridors, and other moist habitats (Katz and Shafroth 2003). Russian olive was originally planted in North America around the early 1900s. Early accounts place these early plantings in New Mexico, Arizona, and Nevada, between 1903 and 1910 by Russian Mennonites who immigrated to the United States and brought the shrub along with them (Stannard et al. 2002). Definitive dates for establishment were recorded in Utah, where tree boring indicated establishment between 1924 and 1929 (Christensen 1963).

Historically, Russian olive was widely planted across many of the western and mid-western states for shelterbelts, as an ornamental, for wildlife/habitat, snowbreaks, and erosion control (Olson and Knopf 1986a, Christensen 1963, Zouhar 2005). Beginning in the early 1930s Russian olive was promoted by many government agencies, in particular the USDA-Soil Conservation Service (SCS), later the USDA-Natural Resource Conservation Service (NRCS). Many agencies promoted Russian planting in dryland arid to semi-arid environments where it was well adapted (Stannard et al. 2002, Zouhar 2005). Widespread plantings occurred across much of the southwestern United States following the advice of local extension services, government agencies, and horticultural specialists of the time (Zouhar 2005, Brock 1998). Until the 1970s Russian olive was one of the few medium height trees commercially available for use in shelterbelts and windbreaks in dryland areas (Stannard et al. 2002). Olson and Knopf (1986b) reviewed the subsidization by local, state, and federal government agencies concerning Russian olive promotion. They found that 16 out of the 17 western states subsidized the sale and distribution of Russian olive saplings, seedlings and cuttings to private land owners. Prices for 100 seedlings in 1985 ranged from \$9.20 in California to \$75.00 in Washington, with an average around \$35.50 for the 17 western United States, where today Russian olive is a major problem. Only one state, Oregon, offered the seeds for free and only one state, Utah, forbade the sale of the seedlings.

A SCS Biologist, A.E. Borell (1951), promoted Russian olive, indicating that the trees provided several benefits for western land owners. First, Russian olive trees were touted as being an excellent candidate for living fences due to their bush-like growth pattern. Second, the trees were promoted for their high quality fruit production and cover

for some 40 different avian and mammalian species. Finally, the trees were promoted as being rugged enough to survive the harsh western landscapes by exhibiting drought tolerance, a wide range of soil habitability ranging from sands to heavy clays, salt tolerance, shade tolerance, resistance to competition from surrounding sod forming grasses and other plants, and a natural ability to form dense thickets (Borell, 1951). These inherent characteristics, which were so valued during Russian olive's promotion, would later serve as the foundation for its resilience in western states, where it is now considered an invasive species.

The first instance of Russian olive escaping cultivation was reported in Utah in 1924 and this species was considered naturalized by 1948 (Stannard et al. 2002, Christensen 1963, Knopf and Olson 1984). Subsequent reports of Russian olive escaping cultivation were provided by Christensen (1963), Nevada (1925), California (1935), Arizona (1941), Idaho (1952), Colorado (1954), New Mexico (1960), and Texas (1960). Today Russian olive is considered naturalized in 17 western states from California to Canada and eastward to the Dakotas, Nebraska, Kansas, Oklahoma, and Texas (Olson and Knopf 1986, Katz and Shafroth 2003). Russian olive has been designated as noxious in three states; Colorado, Wyoming, and New Mexico.

Russian olive is reported to be abundant in the northern Great Plains and much of the west. It is uncommon in southern California, Arizona, Texas, and much of the central Great Plains (Friedman et al. 2005 Katz and Shafroth 2003). Along the east coast Russian olive is present, but it rarely escapes cultivation (Katz and Shafroth 2003). Russian olive is also reported to be rare in Mexico (Katz and Shafroth 2003).

Dispersal

A key factor for a species to become invasive is the capacity of that exotic species to associate with native organisms in mutualistic interactions (e.g. pollination, dispersal) (Bartuszevige and Gorchov, 2006). Dispersal away from the parent plant is a key interaction that increases the inherent spread of an exotic species and may lead it to becoming invasive. Dispersal may also reduce the intra-specific competition and may enhance germination due to passage of the seed through the digestive tract of vertebrates (Robertson et al., 2006). Frugivory provides a strong vector for seed dispersal to new habitats, thereby increasing the opportunity for invasion (Goddard et al., 2009). Passage through the digestive tract can affect seeds in four ways: 1) scarification of the seed coat; 2) removal of germination inhibitors found in the seeds pulp; 3) enhancement of the seeds germination through fecal matter surrounding the seed after defecation; and 4) rendering the seed non-viable through digestion (Robertson et al., 2006). The potential for scarification increasing germination of Russian olive has received little attention in the literature. Heit (1967) recommended that Russian olive seeds be soaked in a concentrated bath of sulfuric acid. Shafroth et al. (1995) acid scarified Russian olive seeds in concentrated sulfuric acid for 1 hour and found a wide range of Russian olive responses with little net mortality. A natural means for acid scarification of the seed coat exists. However, dispersal agents, such as birds, not only digest the seed to scarify it, but transport the seeds to new locations where they can spread and invade new areas.

The scientific literature cites many instances of Russian olive spreading through a potential avian vector and this is primarily linked to its large production of highly nutritious, viable fruits (Kindschy 1998, Stoleson and Finch 2001, Olson and Knopf

1986, Van Dersal 1939, Borell 1951, Evans and Dietz 1974). The key aspect of dispersal is the potential for vertebrates (e.g. avian and mammalian sources) to distribute seeds from a host plant. Van Dersal (1939) showed several species that had been observed to feed upon Russian olive fruits; Eastern and Western American Robins (*Turdus migratorius*), Ring-Necked Pheasants (*Phasianus colchicus*), Sharp-Tailed Grouse (*Tympanuchus phasianellus*), Cedar Waxwing (*Bombycilla cedrorum*), Hungarian Partridge (*Perdix perdix*), Northern Bobwhite (*Colinus virginianus*), Western Evening Grosbeak (*Coccothraustes vespertinus*), Valley Quail (*Callipepla californica*), and the Bohemian Waxwing (*Bombycilla garrulus*). Borell (1951) described 28 more birds, other than those listed by Van Dersal (1939), which feed on Russian olive fruit.

There is no rigorous scientific study to indicate that birds are actually acting as a Russian olive dispersal vector. Many of the citations are eyewitness reports of birds simply observed perching upon Russian olive trees (Olson and Knopf 1984, Olson and Knopf 1986, Stolson and Finch 2001, Borell 1951, Van Dersal 1939). Furthermore, a wide range of species are reported as Russian olive vectors and in particular birds of varying size (ranging from small finches up to large game and waterfowl) and dietary preferences (insectivores, granivores, herbivores, etc) suggest that no rigorous experiments have been conducted to accurately describe avian dispersal – only conjecture. One bird that is continually mentioned in the literature and often reported browsing amongst Russian olive trees in Colorado is the European starling (*Sturnus vulgaris*)

European starlings are invasive birds that were introduced into North America, from 1890 to 1891 in New York City by 100 initial founders (Cabe 1993). These birds

were released intentionally by the Acclimatization Society in an attempt to emulate the world in William Shakespeare's plays. From these initial 100 birds Cabe (1993) estimated that the current North American population of European starlings is above 200 million birds and is steadily increasing. European starlings are described as stocky, rugged, compact passerine, with an easily identifiable glossy/shiny coat of dark iridescent feathers, a short/squared tail, long thin beaks, and long pointed wing tips (Cabe 1993). Native ranges of Starlings stretch throughout much of Eurasia, from Scandinavia to Italy with a western edge somewhere east of Lake Baikal in Russia (Cabe 2003). Coincidentally, this same range overlaps with the natural range of Russian olive; however, there is no information in the literature about feeding choices of European Starlings on trees in their native range. In North America European starlings are typically found in greater abundance in the eastern half of the country due to better feeding/foraging sites and more acceptable nesting locations (Cabe 2003). The western United States has a large annual population of starlings, but their distribution is often interrupted by mountain ranges (Cabe 2003). Starlings are migratory vary their geographic locations during two seasonal migrations (September to December, and again from mid February to March). European Starlings feed upon a wide breadth of foods, the presence of which is seasonally dependent (Cabe 2003, Martinez del Rio et al. 1995, Fischl and Caccamise et al. 1987, Russell 1971). During spring and summer, Starlings are characterized as omnivores, splitting their feeding presence between insects and seeds. Russell (1971) compared the gut contents of 211 birds from across the country and found that 73% of the birds' annual diet consisted of animal material. This was primarily composed of insects, in particular 48% were Orthopterans, 36% Coleopterans,

14% Lepidopterans, and the remainder was a mixture of four other orders. Fischel and Caccamise (1987) observed that of the gut contents of 149 birds were composed of 44.5% plant material, primarily tree (38.1%) and shrub (6.4%) fruits. Contents were also screened for invertebrate components, which were composed of Coleoptera (9.1%), Formicidae (1.2%), Orthoptera (0.2%), Hemiptera (0.1%), Homoptera (<0.1%), and Mollusca (0.7%). During winter months, Starlings supplement their diets by changing their gut morphology to feed on berries, grains, seeds, garbage, livestock feed and many other opportunistic food choices that may present themselves (Cabe 2003). Birds often forage in mixed species flocks with other passerines, ranging from Red-winged Blackbirds (*Agelaius phoeniceus*), Brown-headed Cowbirds (*Molothrus ater*), Common Grackles (*Quiscalus quiscula*), and American Robins (Cabe 2003). Individual birds often require between 7 and 23g of animal based foods (roughly 50 to 60 kcal/day) or between 80 and 100 g of plant material (roughly 80 to 100 kcal/day) to maintain their metabolism and meet their daily dietary needs (Cabe 2003). Kindschy (1998) performed a study in which Russian olive seeds passed through the digestive tracts of a flock of European starlings. One hundred and fifteen excreted Russian olive seeds and 143 whole Russian olive seeds were collected in January after Kindschy observed a large flock of European starlings feeding in a large grove of Russian olive trees. Seeds were kept in their individual groups and sown in two large pots filled with a silty-loam soil. Pots were then placed on a windowsill in direct sunlight. Data were collected on date of germination. The proportion of sprouting seeds to dormant seeds was the same for digested seeds (10.4%) and undigested seeds (9.8%) (Kindschy 1998)

Ecological impacts

Because its native range includes temperate regions of southern Europe and Eurasia, Russian olive is pre-adapted to the climatic conditions of the western United States (Katz and Shafroth 2003). In many western riparian environments where Russian olive has invaded, it is a co-dominant species with the surrounding native plants (Brock 1998). In other areas, it co-dominates with other invasive species forming dense polycultures, or forms dense monocultures of Russian olive trees (Brock 1998). Olson and Knopf (1986) found that infestations of Russian olive may decrease the suitability of a site for germination and establishment of plains cottonwood by altering the soil moisture content. The same authors further speculated that with its continued spread in the western U.S. Russian olive may one day become the most dominant riparian tree (Olson and Knopf 1986). Friedman et al. (2005) concluded that Russian olive was the fifth most prevalent riparian tree in the western United States. In particular, Russian olive is regarded as the poster child for displacement of plains cottonwood (*Populus deltoides* Marsh).

Plains cottonwood is often the dominant riparian tree in much of the Great Plains and western riparian areas (Pearce and Smith 2001, Friedman et al. 2003). Plains cottonwood can grow upwards of 30 meters tall and have a trunk diameter between 1 to 2 meters (Lesica and Miles 1999). Female cottonwood trees produce millions of wind dispersed seeds in early springtime. Seeds have very specific germination requirements (moist, mineral rich soils that are disturbed) thus, limiting their spread into unsuitable habitats (Lesica and Miles 1999). Seeds require full sunlight, as they are shade intolerant (Lesica and Miles 1999). Recruitment of cottonwood seedlings is relatively low; successful recruitment may occur 1 out of every 5 to 10 years (Pearce and Smith 2001).

The concern is that as older stands of cottonwood die, under stories that have become infested with Russian olive trees will thrive and push out native late seral species. This process would thus prevent further cottonwood recruitment and successful establishment (Lesica and Miles 1999).

Russian olive can affect both early and late successional stages. In early succession, Russian olive can quickly colonize disturbed sites due to the nitrogen fixing associations that the trees can develop (Zouhar 2005). Nitrogen content in Russian olive leaves were calculated to be between 1.6 to 3.3% (Katz and Shafroth 2003, Royer et al. 1999). This high nitrogen level may represent a significant source of nitrogen into limited, disturbed systems (Katz and Shafroth 2003, Bermudez de Castro et al. 1990). In late successional habitats the large seed size provides Russian olive much needed resources that allow seedlings to establish under dense canopies (Zouhar 2005). The large seed size also allows Russian olive to establish at anytime during the growing season, unlike other riparian species. In many western riparian corridors, pioneer or early seral species, such as cottonwood and many willow species, depend upon a continual disturbance regime and seasonal floods to create bare areas where their light, wind-dispersed seeds may colonize (Katz and Shafroth 2003).

Implications of these future invasions of riparian environments by Russian olive include displacement and death of large fauna. Knopf and Olson (1984) concluded that Russian olive may increase lateral and horizontal infrastructures of many riparian corridors, by augmenting the intermediate height structure. Katz and Shafroth (2003) concluded that Russian olive groves create a new niche by introducing the intermediate height to vertical structure limited riparian corridors. This new intermediate height niche

can have both positive and negative consequences for birds. Russian olive can provide an “intermediate” height structure in often predominantly “tall” height structured riparian environments. This often attracts bird species that would not otherwise occur in these environments and offers a large crop of edible fruits/seeds during the late summer through winter months (Shafroth et al. 1995, Knopf and Olson 1984, Borell 1951). Knopf and Olson (1984) found that Mourning Doves (*Zenaida macroura*) and Yellow-breasted Chats (*Icteria virens*) were abnormally abundant in Russian olive stands, compared to native upland and riparian sites. Knopf and Olson observed that Russian olive infested sites provided excellent foraging habitats for birds including Western Tanagers (*Piranga ludoviciana*) and Evening Grosbeaks, that typically nest in non-riparian environments.

Research has indicated, however, that dense stands of Russian olive trees may in fact decrease biodiversity by limiting the number of invertebrates and by competing with native plants. Brown (1990) observed that Russian olive stands had a decreased number of arthropod species when compared to other environments. Monotypic stands of Russian olive supported less biodiversity than stands that were intermixed or stands that were absent of Russian olive (Brown 1990). These observations are backed up by other papers, indicating that Russian olive trees decrease the overall avian and mammalian species richness and dominate areas where “Tall” height structure trees are absent or killed off by competition (Olson and Knopf 1986, Gazda et al. 2002). Russian olives also locally inhibit seedling establishment of native riparian species, alter successional processes to allow non-native species avenues for establishment, and alter nutrient and water regimes (Zouhar 2005, Shafroth et al. 1995, Knopf and Olson 1984). Gazda et al.

(2002) found that duck nest success was linked to the density of Russian olive trees, tending towards lower success with increased density.

Management options

Russian olive eradication is often highly impractical and costly (Stannard et al. 2002). There are currently no biological control arthropods available for Russian olive control. Several bacterial infections have been reported in the literature, as injuring Russian olive trees and are thus available as bio-control agents. Canker causing bacteria that can infect Russian olive include *Tubercularia ulmea*, *Phomopsis arnoldiae* and *P. elaeagni*. Bacterial infections can cause stem death, branch death, and whole tree death over time (Stannard et al. 2002). Infections of the canker causing bacteria may also exude a gum like substance at the sites of infection. This gum is typically amber in color and resembles a large round ball. Common to exudates, these gums may form nodule clusters that may darken and crust over in cool wet weather (Stannard et al. 2002). While these agents can be used for control, their release has not been attempted on broad scales and there is no information in the literature to determine their effectiveness.

Additional physical/mechanical management options such as mowing and removal are appropriate for smaller trees with trunk diameters less than 8 inches. However, there are many instances in the literature indicating excessive re-sprouting of trees that received no other management except mowing or removal (Caplan 2002, Elden and Crowder 2007). To overcome the capability of the trees to resprout, physical removal usually is combined with herbicide applications to provide sufficient, long lasting control.

Russian olive trees are reported to be sensitive to 2,4-D ester, triclopyr ester (Garlon 4), triclopyr amine (Garlon 3A), imazapyr (Habitat, Arsenal), glyphosate (Rodeo, Roundup), and picloram (Tordon 22k) (Stannard et al. 2002, Zouhar 2005). Several application methods have been used effectively. These methods will be discussed below:

Foliar

Foliar applications of herbicides can be effective against Russian olive trees if they are applied multiple times during the growing season. Successful herbicides against Russian olive using foliar applications include glyphosate, imazapyr, 2,4-D, triclopyr ester, 2,4-D + triclopyr ester (Crossbow) (Creech and Rafferty 2007, Uhing 2008, Zouhar 2005). The potential risks from foliar applications include off target damage from multiple applications over several years, difficulty using herbicides in riparian areas, long term control breakdown, and potential non-target effects due to drift or overspraying (Zouhar 2005, Tu 2003).

Bovey (1965) conducted aerial applications of the auxinic herbicides 2,4-D, 2,4,5-T, and 2,4,5-TP (Silvex) in three different experiments. In the first, herbicides were aerially applied using a helicopter on 1 acre plots. Treatments included Silvex (1 and 2 lb active ingredient (ai)/A), 2,4-D (2 lb ai/A), 1:1 2,4-D + Silvex (2 lb ai/A), and a 1:1 2,4-D + 2,4,5-T (2 and 4 lb ai/A). A 2:3 oil/water solution was used for all treatments and applied at 5 gpa in the early summer. Treatments were reapplied to regrowth 2 years later. Results 3 years after application showed that Silvex controlled 100% and 70% of Russian olive at 1 and 2 lb/A, respectively; 20% control from 2,4-D; 100% control from both rates of 2,4-D + 2,4,5-T; and 70% control from 2,4-D + Silvex. .

The researchers used similar methodology and treatments in the second experiment (Silvex, 2,4-D, and 1:1 2,4-D + 2,4,5-T all at 2 lb ai/A). The carrier was changed to include only diesel oil. Results 2 years after treatment suggested poor control from Silvex (20%), moderate control from 2,4-D (70%), and 100% control of Russian olive from 2,4-D + 2,4,5- T. In the third experiment, a standard treatment of the 1:1 ratio of 2,4-D + 2,4,5-T at 2 lb /A was tested in different carriers and volumes that included water alone, water plus a surfactant (unspecified), or diesel fuel alone at 5 and 10 gpa. Results 2 years after treatment included 100% control from the herbicide + water or water plus surfactant carriers, and 95% control from the herbicide plus diesel carrier all at 5 gpa. When the carrier was increased to 10 gpa, 85% of Russian olive were controlled by the herbicide plus water carrier, 95% control from the water plus surfactant, and 100% control from the herbicide plus diesel carrier.

Ohlenbusch and Ritty (1978) applied 1 gallon of either 2,4,5-T (2 lb ai/A), Silvex (2 lb ai/A), dicamba (2 lb ai/A), picloram (0.5 lb ai/A), and glyphosate (1% and 2% v/v) with a 90% water:10% diesel fuel mixture to Russian olive trees. Data were collected the following year and all treatments except the glyphosate resulted in total root kill. Both glyphosate mixtures resulted in extensive collateral damage to the underlying herbaceous vegetation. Of the herbicides evaluated in the above four experiments, only dicamba, picloram, and 2,4-D remain available for use.

Cut-stump

Cut-stump applications of herbicides to the trunk surface are often highly effective for Russian olive control (Creech and Rafferty 2007, Zouhar 2005, Tu 2003,

Parker and Williamson 2003). In cut stump applications trees are cut down as low as possible to the soil surface using either chainsaws or other forms of mechanical removal (e.g. industrial loppers, etc). Cuts to the trunk are horizontal to maximize the surface area and prevent runoff of the herbicide solutions during application (Parker and Williamson 2003). Herbicides are applied to the cut surface of the stumps, primarily along the cambial layer, almost immediately or within 5 minutes (Tu 2003, Parker and Williamson 2003). It is essential that herbicides used for cut-stump treatments be applied within 5 minutes of exposing the cut surface to avoid suberization of the trunk and prevention of herbicide uptake. Herbicides effective for cut stump applications include triclopyr ester (applied undiluted to cut surfaces), triclopyr amine, imazapyr (applied undiluted to the cut surface) (Creech and Rafferty 2007, Uhing 2008, Zouhar 2005).

Caplan (2002) performed cut-stump applications in New Mexico along the Rio Grande River. Herbicides were applied within 5 minutes of cutting using a 50% mixture of triclopyr amine plus water. The following summer, numerous resprouts were observed on the site; trees that were less than 8 inches in diameter had few resprouts, but the stumps that were larger and sprayed had a high proportion of resprouts. It was determined that the 50% mixture of triclopyr amine was inadequate for controlling the trees. Subsequently, trees were re-sprayed with a 25% mixture of triclopyr amine over the next 3 years to achieve adequate control.

Edelen and Crowder (1997) performed mechanical removal of the top growth of Russian olive trees in Washington during July and August of 1996. Stumps were treated with two rates of imazapyr (either a 2% or a 4% solution). Initial signs of herbicide injury were reported within 3 weeks of application; 75% of the trees sprayed with the 4%

solution were reported as having observable damage, whereas only 60% of the trees showed injury from the 2% solution.

Basal Bark

Foliar treatments often cause extensive collateral damage and cut-stump treatments are very labor intensive. Thus, a need for an application method that decreased these negative aspects was needed and basal bark methodology was developed. In basal bark applications, herbicides are tank mixed with an oil-based carrier. Ohlenbusch and Ritty (1978) applied 2,4,5-T (5lb), Silvex (5 lb), Dicamba (5 lb), and triclopyr (5lb), each mixed with 100 gal of diesel fuel to Russian Olive trees. Data was collected 1 YAT and it was concluded that all treatments achieved 100% control, except for the Dicamba, which was incompatible with the diesel fuel and offered 0% control. While these applications were originally successful and demonstrated an avenue towards a new technique, environmental concerns have prevented their application.

Currently, vegetable oil-based basal oil (JLB Oil) or petroleum-based basal oil (Bark Oil Blue LT) are typically used as carriers for basal bark applied herbicides (Parker and Williamson 2003). Herbicides shown to be effective for basal bark applications to control Russian olive include 2,4-D + triclopyr ester, and triclopyr ester (Creech and Rafferty 2007, Parker and Williamson 2003). Applications are made to the base of trees from the soil surface up 12 inches or more along the trunk (Parker and Williamson 2003). The oil in the solution provides two essential effects: 1) aids in penetration of bark by the herbicide; and 2) allows the herbicide to adhere to the tree surface and wrap around the entire trunk without spraying all sides (Parker and Williamson 2003). Stannard et al.

(2002) reported that triclopyr ester was highly effective when used as basal bark applications.

Hack and squirt

Similar to basal bark applications, hack and squirt methodology allows dead trees to remain, which is valuable to maintain nesting and brood cover for many avian species and limits collateral damage that often occurs from cut stump and foliar applications (Clubine 2008). Hack and squirt applications have shown excellent control of mature Russian olive trees (Tu 2003).

In the hack and squirt technique a hand held hatchet is used to create a wound into the cambial layer creating a 1 to 1.25 inch “cup” in which undiluted herbicides are applied (Clubine 2008). The wound created by the hack is to provide direct avenue in to the phloem tissue of the plant, further increasing the efficacy of transport of herbicides into the trees through translocation (Parker and Williamson 2003). Moorhead (2003) recommends that hack and squirt applications be made using a technique which calls for one hack for every 3 inches of trunk diameter, with 1 ml of herbicide solution applied to each wound. Stannard et al. (2002) reported that both imazapyr and glyphosate can be applied, undiluted, into frill cuts and provide excellent control of Russian olive trees. Parker and Williamson (2003) reported that 50% mixtures of triclopyr amine in water and mixtures of 50% triclopyr amine + 3 ounces of imazapyr offer good control of Russian olive trees using this technique.

Aminocyclopyrachlor:

Field trials are now being conducted by private industry and university weed scientists on DuPont Crop Protection's new chemistry aminocyclopyrachlor (6-amino-5chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid or DPX-MAT 28 for the free acid formulation and 6-amino-5chloro-2-cyclopropyl-4-pyrimidin methyl ester or DPX-KJM 44 for the methyl ester formulation) (Bukun et al. 2010). DPX-KJM 44 was the primary product in the early field testing stages, but is being replaced by DPX-MAT 28 as the product progresses forward.

Aminocyclopyrachlor is being developed for rangeland, pasture, forestry, non-cropland, rights-of-way, industrial sites, and natural areas (Bukun et al. 2010, Sensenman 2007, Turner et al. 2009). The results to date indicate excellent commercial control of many noxious and invasive weeds such as Russian knapweed (*Acroptilon repens* L. DC.), kochia (*Kochia scoparia* L.), leafy spurge (*Euphorbia esula* L.), field bindweed (*Convolvulus arvensis* L.), musk thistle (*Carduus nutans* L.), Canada thistle (*Cirsium arvense* L.) trumpetcreeper (*Campsis radican* (L.) Seem. ex Bureau), mugwort (*Artemisia vulgaris* L.), buckhorn plantain (*Plantago lanceolata* L.), common ragweed (*Ambrosia artemisiifolia* L.), horsenettle (*Solanum carolinense* L.), red clover (*Trifolium pretense* L.) crownvetch (*Coronilla varia* L. Lassen), and horseweed/marestail (*Conyza canadensis* L. Cronquist) (E.I. DuPont, 2009, Armel et al. 2009, Blair and Lowe 2009, Evans et al. 2009, Montgomery et al. 2009). Field trials have confirmed that aminocyclopyrachlor has a response pattern similar to many of the other synthetic auxin herbicides (Claus et al. 2008, Bukun et al. 2010).

Aminocyclopyrachlor is in a new family of chemistry called the pyrimidine carboxylic acids under the synthetic-auxic herbicides mode of action (E.I. DuPont 2009,

Bukun et al. 2010). Structurally, aminocyclopyrachlor is very similar to the pyridine carboxylic herbicides, such as picrolam (Tordon 22k), aminopyralid (Milestone), and clopyralid (Transline) (Sensenman 2007). However, the aminocyclopyrachlor molecule differs in that it possesses an additional nitrogen in its heterocyclic carbon ring structure and that there is a cyclopropyl side chain (Bukun et al. 2010).

Chemically, the free acid aminocyclopyrachlor (DPX-MAT28) has a pKa dissociation constant of 4.65 making it fairly phloem mobile. Based on previous research aminocyclopyrachlor translocates very rapidly to meristematic regions of the plant, where it can act as an auxin mimic (E.I. DuPont 2009). Aminocyclopyrachlor has a log octanol-water partitioning coefficient (Log Kow) ranging between -2.48 and -1.12, based upon pH 7 and 4, respectively (E.I. DuPont 2009, Bukun et al. 2010). Its Log Kow makes aminocyclopyrachlor fairly water soluble and thus a hydrophilic herbicide. Its half-life in water has been calculated for a pHs of 4, 7, and 9 and at each pH, aminocyclopyrachlor is stable (E.I. DuPont 2009). Aminocyclopyrachlor (DPX-MAT 28) has been shown to be active in the soil and can be taken up by plant roots (E.I. DuPont 2009, Lindenmayer et al. 2009). Degradation and mineralization by microbes has been documented (E.I. Dupont 2009). Aminocyclopyrachlor is considered a relatively “safe” herbicide from a toxicological perspective. Oral and dermal LD₅₀ values have been determined at >5000 mg/kg; it is considered a mild eye irritant and a non-irritant of skin for mammals (E.I. DuPont 2009). Avian oral LD₅₀ values have been determined at >2075 mg ae/kg body weight, and freshwater fish toxicity is >122 mg ae/L (E.I. DuPont 2009).

In 2007 research conducted by DuPont and the University of Nebraska demonstrated that aminocyclopyrachlor (DPX-KJM 44) applied as a foliar herbicide offered excellent control of Russian olive trees within 1 year of application (Nielson and Wilson 2009). The trees became defoliated and did not produce seeds. Edwards et al. (2009) performed a similar experiment in the fall of 2007 using the same experimental design used in previous research to assess the possibility of a seasonal variation in control. Four rates of aminocyclopyrachlor (DPX KJM-44) (2, 4, 6, 8 oz ai/A), were applied to two size classes (e.g. trees <10 ft tall, and trees >10 ft tall).

Data from the field indicated that approximately 90-100% of Russian olive was controlled at the 8 oz ai/A rate, with control progressively decreased as rate decreased (Edwards et al. 2009). Field observations from our experiment also indicate that complete coverage is essential for optimum control. Observations also revealed that as overall size of the Russian olive trees increased, so did recovery and re-growth, suggesting larger trees display decreased susceptibility to herbicides. Edwards observed that trees that were less than 10 feet tall were more susceptible to the herbicide and trees that were taller than 10 feet re-grew and developed new foliage.

These observations strongly suggest that tree size may influence Russian olive control and this aspect warrants further study. The effect of coverage, however, also should be addressed as an experimental component to define its influence on control. The observations of both university and DuPont Crop Protection representatives were promising enough to encourage continued research into the effects of aminocyclopyrachlor (DPX-MAT28) on Russian olive. The goal is to develop a new

herbicide to control Russian olive by public and private land owners seeking options for long term control of ever expanding stands of Russian olive.

LITERATURE CITED

- Armel, G. R., W. E. Klingeman, P. C. Flanagan, G. K. Breeden, and M. Halcomb. 2009. Comparisons of the experimental herbicide DPX-KJM44 with aminopyralid for control of key invasive weeds in Tennessee. Abstract 410 in Proceedings of the 49th annual WSSA meeting in Orlando, FL.
- Bartuszevige, A.M., and D.L. Gorchov. 2006. The relative importance of landscape and community features in the invasion of an exotic shrub in a fragmented landscape. *Ecography*. 29(2): 213-222.
- Bermudez de Castro, F., Y. Aranda, and M.F. Schmitz. 1990. Acetylene-Reducing Activity and Nitrogen Inputs in a Bluff of *Elaeagnus angustifolia* L. *Orsis*. 5: 85-89.
- Blair, M. and, Lowe, Z. 2009. Evaluation of KJM-44 for marestail (*Conyza canadensis*) and total vegetation control. Abstract 406 in Proceedings of the 49th annual WSSA meeting in Orlando, FL.
- Borell, A. E. 1951. Russian olive as a Wildlife Food. *Journal of Wildlife Management* 15: 109-110.
- Bovey, R.W. 1965. Control of Russian olive by Aerial Application of Herbicides. *Journal of Range Management*. 18 (4): 194-195.
- Brock, J.H. 1998. Invasion, Ecology, and Management of *Elaeagnus angustifolia* (Russian olive) in the Southwestern United States of America. 123-136. In U. Starfinger, K. Edwards, I. Kowarik, and M. Williamson. *Plant Invasions: Ecological Mechanisms and Human Responses*. Backhuys Publishers, Leiden, The Netherlands
- Brown, C.R. 1990. Avian Use of Native and Exotic Riparian Habitats on the Snake River, Idaho. MA Thesis Colorado State University, Ft. Collins, Co.
- Bukun, B., R.B. Lindenmayer, S.J. Nissen, P. Westra, D.L. Shaner and G. Brunk. 2010. Absorption and Translocation of Aminocyclopyrachlor and Aminocyclopyrachlor Methyl-Ester in Canada Thistle (*Cirsium arvense*). *Weed Science*. 58: 96-102
- Cabe, P.R. 1993. European Starling (*Sturnus vulgaris*). *The Birds of North America*. 48: 1-23.
- Caplan, T. 2002. Controlling Russian olive within Cottonwood Gallery Forests along the Middle Rio Grande Floodplain (New Mexico). *Ecological Restoration*. 20 (2): 138-139.
- Christensen, E.M. 1963. Naturalization of Russian olive (*Elaeagnus angustifolia* L.) in Utah. *American Midland Naturalist*. 70-1: 133-137.

- Claus, J., R. Turner, G. Armel, and M. Holliday. 2008. DuPont aminocyclopyrachlor (Proposed Common Name) (DPX-MAT28/KJM44) herbicide for use in turf, IWC, bare-ground and brush markets. Proceedings of the International Weed Science Society of America. 5:654.
- Clubine, S. 2008. Hack and Squirt Verses Basal Bark for Controlling Woody Encroachment of Grassland Quail Habitat. Proceedings of the Joint Meeting for the Society for Range Management and American Forage and Grassland Council, Louisville, KY.
- Creech, E., and D. Rafferty. 2007. Identification and Management of Russian olive. University of Nevada Cooperative Extension Fact Sheet 07-39.
- Edelen, W.J., and W.A. Crowder. 1997. Russian olive (*Elaeagnus angustifolia*) Control Experiment Underway (Washington). Restoration & Management Notes. 15 (2): 198-199.
- Edwards, R.J., J. Sebastain, K.G. Beck. 2009. Variability of Russian Olive Control Using Herbicides. Abstract 7 in Proceedings of the 62nd annual WSSA meeting in Albuquerque, NM.
- E.I. DuPont de Nemours and Company. 2009. DuPont DPX-MAT 28 Herbicide Technical Bulletin.
- Evans, C. C., D. P. Montgomery, and D. L. Martin. 2009. Musk thistle control on Oklahoma highway rights-of-way with DPX-KJM44. Abstract 420 in Proceedings of the 49th annual WSSA meeting in Orlando, FL.
- Evens, K.E., and D.R. Dietz. 1974. Nutritional energetic of sharp-tailed grouse during winter. Journal of Wildlife Management. 38-4:622-629.
- Fischl, J., and D.F. Caccamise. 1987. Relationships of Diet and Roosting Behavior in the European Starling. American Midland Naturalist. 117 (2): 395-404.
- Friedman, J. M., G. T. Auble, P. B. Shafroth, M. L. Scott, M. F. Merigliano, M. D. Freehling, and E. R. Griffin. 2005. Dominance of Non-Native Riparian Trees in Western USA. Biological Invasions 7: 747-751.
- Gazda, R.J., R.R. Medinger, I.J. Ball, J.W. Connelly. 2002. Relationship between Russian olive and Duck Nest Success in Southeastern Idaho. Wildlife Society Bulletin. 30 (2): 337-344.
- Goddard, R.H., Webster, T.M., Carter, R., and Grey, T.L. 2009. Resistance of Benghal dayflower (*Commelina benghalensis*) seeds to harsh environments and the implications for dispersal by Mourning doves (*Zenaida macroura*) in Georgia, U.S.A. Weed Science 57: 603-612.

- Hamilton D.F., and Carpenter, P.L. 1975. Regulation of seed dormancy in *Elaeagnus angustifolia* by Endogenous Growth Substance. *Canadian Journal of Botany* 54: 1068-1073.
- Hazelton, P.K., R.J. Robel, and A.D. Dayton. 1984. Preferences and Influence of Paired Food Items on Energy Intake of American Robins and Gray Catbirds. *Journal of Wildlife Management*. 48: 198-202.
- Heit, C.E. 1967. Propagation from seed part 6: Hardseededness-a critical factor. *American nurseryman* 125: 10-12, 88-96.
- Hogue, E.J., and L.J. LaCroix. 1970. Seed Dormancy of Russian olive (*Elaeagnus angustifolia* L.). *Journal of American Society for Horticultural Science*. 95(4): 449-452.
- Jinks, R.L., and Ciccarese, L. 1997. Effects of Soaking, Washing and Warm Pretreatment on the Germination of Russian olive and Autumn Olive Seeds. *Tree Planters Notes*. Winter/spring 1997: 18-23.
- Katz, G L., and P. B. Shafroth. 2003. Biology, Ecology and Management of *Elaeagnus angustifolia* L. *Wetlands*. 23: 763-777.
- Kendeigh, S.C. 1969. Tolerance of Cold and Bergmanns Rule. *The Auk*. 86: 13-25.
- Kindschy, Robert R. 1998. European Starlings Disseminate Viable Russian-Olive Seeds. *Northwestern Naturalist*. 79: 119-20.
- Knopf, F.L. 1986. Changing Landscapes and the cosmopolitanism of the eastern Colorado avifauna. *Wildlife society bulletin*. 14-2: 132-142.
- Lessica, P., and S. Miles. 1999. Russian olive invasion into Cottonwood Forests along a Regulated River in North Central Montana. *Canadian Journal of Botany*. 77: 1077-1083.
- Lindenmayer, B., P. Westra, and G. Brunk. 2009. Soil interactions with DPX-KJM44 and DPX-MAT28. Abstract 515 in Proceedings of the 49th annual WSSA meeting in Orlando, FL.
- Martinez del Rio, C., K.E. Brugger, J.L. Rios, M.E. Vergara, and M. Witmer. 1995. An Experimental and Comparative Study of Dietary Modulation of Intestinal Enzymes in European Starlings (*Sturnus vulgaris*). *Physiological Zoology*. 68(3): 490-511.
- Montgomery, D., C. Evans, and D. Martin. 2009. Control of Kochia with DPX-KJM44 along Oklahoma Highway Rights-of-way. Abstract 493 in Proceedings of the 49th annual WSSA meeting in Orlando, FL.

- Moorhead, D. 2003. Wide-space injection with Arsenal AC herbicide for control of undesirable hardwood stems. Available at: <http://www.bugwood.org/weeds/arsenal.html>
- Nielsen, P., and R. G. Wilson. 2009. Cut Stump Herbicide Applications on Russian Olive. Abstract 46 in Proceedings of the 62nd annual WSSA meeting in Albuquerque, NM.
- Ohlenbusch, P.D., and P.M. Ritty. 1978. Russian olive Control: A Preliminary Look. Proceedings of the North Central Weed Control Conference. 33: 132.
- Olson, D.F., and J.R. Barbour. 2002. *Elaeagnus L.*USFS. Available at: <http://www.nsl.fs.fed.us/wpsm/Elaeagnus.pdf>
- Olson, T. E., and F. L. Knopf. 1984. Naturalization of Russian-olive implications to rocky mountain wildlife. Wildlife Society Bulletin. 12: 289-298.
- Olson, T. E., and F. L. Knopf. 1986a. Naturalization of Russian-Olive in the Western United States. Western journal of applied forestry 1: 65-69.
- Olson, T. E., and F. L. Knopf. 1986b. Agency Subsidization of a Rapidly Spreading Exotic. Wildlife Society Bulletin. 14: 492-493.
- Parker D., and M. Williamson. 2003. Low-Impact, Selective Herbicide Application for Control of Exotic Trees in Riparian Areas: Salt Cedar, Russian olive and Siberian Elm. United States Department of Agriculture: Forests Service-Southwest region.
- Pearce, C.M., and D.G. Smith. 2001. Plains Cottonwood's Last Stand: Can it survive Invasion of Russian olive onto the Milk River, Montana Floodplain? Environmental Management. 28(5): 623-637.
- Riffle, J.W. 1977. First Report of Vesicular-Arbuscular Mycorrhizae on *Elaeagnus angustifolia*. Mycologia. 69 (6): 1200-1203
- Robertson, A.W., Trass, A., Ladley J.J and Kelly, D. 2006. Assessing the benefits of frugivory for seed germination: the importance of the deinhibition effect. Functional Ecology. 20: 58-66.
- Royer, T.V., M.T. Monaghan, M.G. Wayne. 1999. Processing of Native and Exotic Leaf Litter in Two Idaho Streams. Hydrobiologia. 400: 123-128.
- Russell, D.N. 1971. Food Habits of the Starling in Eastern Texas. The Condor. 73(3): 369-372.
- Sensenman, S.A. 2007. Herbicide Handbook 9th edition. Lawrence, KS: Weed Science Society of America

- Shafroth, P.B, G.T. Auble, and M.L.Scott. 1995. Germination and establishment of native plains cottonwood (*Populous deltoides* marshall subsp. *monilifera*) and exotic russian-olive (*Elaeagnus angustifolia* L.). *Conservation Biology*. 9: 1169-1175.
- Stannard, M., D. Ogle, L. Holzworth, J Scianna, and E Suleaf. HISTORY, BIOLOGY, ECOLOGY, SUPPRESSION OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA* L.)L.)L.). USDA, NRCS. Boise, ID: USDA, 2002. 1-14.
- Stoleson, Scott H., and Deborah M. Finch. 2001. Breeding Bird Use of and Nesting Success in Exotic Russian olive in New Mexico. *The Wilson Bulletin*. 113: 452-55.
- Tu, M. 2003. Element Stewardship Abstract for *Elaeagnus angustifolia* L. or Russian olive, Oleaster. The Nature Conservancy. Available at:
<http://www.imapinvasives.org/GIST/ESA/esapages/documnts/elaeang.pdf>
- Turner, R.G., J.S. Claus, E. Hidalgo, M.J. Holliday, and G.R. Armel. 2009. Technical introduction of the new DuPont vegetation management herbicide aminocyclopyrachlor. *Weed Science Society of America Abstract*. 49:405.
- Uhing, K. 2008. Russian olive Identification and Management. Colorado Department of Agriculture-Conservation Service division Noxious weed species List B fact sheet.
- Van Dersal, and William R. 1939. Birds that feed on Russian olive. *The Auk*. 56: 483-84.
- Zouhar, K. 2005. *Elaeagnus angustifolia*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/>

Chapter 2: Seasonal Control of Russian olive Using Aminocyclopyrachlor as a Cut Stump Application

ABSTRACT

Field trials were conducted on Russian olive trees testing aminocyclopyrachlor (DPX-MAT 28 SL) efficacy for cut stump applications. Trees were cut down and herbicides applied using a CO₂ backpack sprayer with a 40-03E single nozzle boom and applied at 1 fluid oz per inch of trunk diameter to the entire stump. Aminocyclopyrachlor was applied at 1, 2.5, 5% v/v and compared to 100% Bark Oil Blue LT (petroleum based carrier), 30% v/v triclopyr ester and a no herbicide control. All treatments were mixed with Bark Oil Blue LT as a carrier. The experiment was designed as a RCB, with 12 replications (one tree per replicate) and conducted at two sites to be repeated in space (Louisville, Colorado and Haigler, Nebraska). Visual estimates of control were conducted 1 YAT based on a binomial scale for dead trees and living trees. For living trees, the number of re-grown shoots and height of shoots were also determined. Data were subjected to analysis of variance and means separated by LSD ($\alpha= 0.05$). There were no statistical differences between our two sites so data were pooled for analysis. Herbicide treatments controlled Russian olive similarly (83-100% control) and were more effective than all but the winter-applied 100% Bark Oil Blue LT (83% control). All herbicides and the Bark Oil Blue LT treatments caused a calculated regrowth factor about 80% less than the non-herbicide control.

INTRODUCTION

Since its introduction into the United States from Eurasia in the early 1900s Russian olive (*Elaeagnus angustifolia* L.) has escaped from being an ornamental and spread across many western habitats (Friedman et al. 2005, Zouhar 2005, Shafroth et al. 1995, Katz and Shafroth 2003). Russian olive trees were commonly planted in cities and yards, and were subsidized by states and the federal government to be used for hedge plants and windbreaks (Brock 1998, Christensen 1963, Olson and Knopf 1986, Stannard et al. 2002,). Between 1941 and 1948 the first evidence of Russian olive trees escaping cultivation were reported in Utah, Nevada, and Arizona (Christensen 1963, Brock 1998). Within 100 years of its arrival Russian olive became the fifth most dominant woody riparian species in the western United States (Friedman et al. 2005). Russian olive is currently found in numerous habitats ranging from moist riparian corridors, to prairies (both tallgrass and shortgrass), and to dry deserts (Zouhar 2005).

Russian olive is a member of the Elaeagnaceae with close relatives in the western United States including silverberry (*Elaeagnus commutate* Bernh. Ex Rydb), buffalo berry (*Shepherdia Canadensis*(L.) Nutt.) and autumn olive (*Elaeagnus umbellate* Thunb) (Katz and Shafroth 2003). Russian olive is a perennial tree or a multi-stemmed shrub, most commonly with reddish bark and sharp thorns along its branches (Zouhar 2005). Trees grow upwards of 40 feet tall and trunks may reach 20 inches in diameter (Stannard et al. 2002, Zouhar 2005). Russian olive leaves are lanceolate and pale green in color with trees producing small yellow flowers in the late spring/early summer (Zouhar 2005). These flowers produce a drupe like fruit that may be spread by birds and other small mammals (Jinks and Ciccarese 1997, Zouhar 2005, Kindschy 1998, Stoleson and Finch

2001, Olson and Knopf 1986, Van Dersal 1939, Borell 1951, Evans and Dietz 1974). Russian olive trees are saline tolerant, drought tolerant, and form actinorhizal associations with the bacterium *Frankia* spp., to carry out nitrogen fixation (Brock 1998, Olson and Knopf 1986, Stannard et al. 2002, Zouhar 2005).

Herbicide applications to the cut trunk surface are typically highly effective to control Russian olive (Caplan 2002, Creech and Rafferty 2007, Edelen and Crowder 1997, Parker and Williamson 2003, Tu 2003, Zouhar 2005). The combination of physical removal of the top portion of the tree, followed by herbicide applications result in the maximum level of Russian olive control (Parker and Williamson 2003). In cut stump applications, trees are cut as close to the soil surface as possible using either chainsaws or other forms of mechanical removal (e.g. industrial loppers, etc). Cuts to the trunk are made as level as possible to maximize the surface area and prevent herbicide runoff during application (Parker and Williamson 2003). Herbicides are applied to the cut surface, primarily along the cambial layer, within 5 minutes to avoid suberization of the exposed tissue (Tu 2003, Parker and Williamson 2003). Herbicides that are effective for cut stump applications include triclopyr ester (Garlon 4), triclopyr amine (Garlon 3A), and imazapyr (Habitat or Arsenal) (Creech and Rafferty 2007, Uhing 2008, Zouhar 2005).

Caplan (2002) performed cut stump applications in New Mexico along the Rio Grande River. Russian olive trees were sawed down and treated within 5 minutes of cutting using a 50% mixture of triclopyr ester and water. The following summer numerous resprouts were observed from trees that were over 8 inches in diameter. As a result of the observed regrowth, researchers concluded that the 50% mixture of triclopyr

ester was inadequate for controlling the trees. Subsequent re-spraying of the foliar regrowth during with a 25% mixture of triclopyr ester with occurred over a 3-year period fell just short of 100% control of all regrowth.

Edelen and Crowder (1997) performed mechanical removal of Russian olive top growth in Washington during July and August of 1996. Stumps were treated with two rates of imazapyr (either a 2% or a 4% solution). There was no information given in the report regarding sample size, application methodology, or inclusion of a carrier. Initial signs of herbicide injury were reported within 3 weeks of application. They reported that 75% of the trees receiving the 4% solution had adequate control, but required future retreatment to provide long lasting control. Similarly, Bossard et al. (2000) found that applications of 5 to 10 ml of undiluted glyphosate can be applied immediately to the cambial layer to provide adequate control during the first year, followed by successive years of monitoring for regrowth and subsequent retreatment.

Field trials are now being conducted on DuPont Crop Protection's new chemistry aminocyclopyrachlor (6-amino-5chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid or DPX-MAT 28 for the free acid formulation and 6-amino-5chloro-2-cyclopropyl-4-pyrimidin methyl ester DPX-KJM 44 for the methyl ester formulation) by private industry and university weed scientists (Bukun et al. 2010). The aminocyclopyrachlor molecule has shown promise in the control of many noxious and invasive weeds (E.I. DuPont, 2009). Aminocyclopyrachlor is being targeted for applications on rangelands, pastures, forestry, non-cropland areas, rights-of-ways, industrial areas, and natural areas (Bukun et al. 2010, Sensenman 2007, Turner et al.. 2009).

Aminocyclopyrachlor (DPX-MAT 28) is in a new family of chemistry called the pyrimidine carboxylic acids under the synthetic-auxic herbicides mode of action (Bukun et al. 2008, Sensenman 2007). Structurally, aminocyclopyrachlor is very similar to the pyridine carboxylic herbicides, such as picloram, (Tordon 22K), aminopyralid, (Milestone) and clopyralid (Transline) (Sensenman 2007). However, the aminocyclopyrachlor molecule differs from these in that it possesses additional nitrogen in its heterocyclic carbon ring structure and a cyclopropyl side chain (Bukun et al. 2010).

The specific objectives of our research were to investigate the possibilities for controlling Russian olive trees using a cut stump application with aminocyclopyrachlor. Our first null hypothesis would be Ho1: control of Russian olive from aminocyclopyrachlor is not comparable to the industry standard triclopyr ester as a cut stump application. Our first alternate hypothesis would be Ha1: control of Russian olive from aminocyclopyrachlor is comparable to the industry standard triclopyr ester as a cut stump application. Our second null hypothesis would be Ho2: season of application did not influence control efficacy among the herbicide treatments. Our second alternate hypothesis would be Ha2: season of application influenced control efficacy among herbicide treatments. The overall objectives of our research were to: 1) determine the effective use rates of aminocyclopyrachlor to achieve acceptable control of Russian olive; and 2) determine the influence application timing.

MATERIALS AND METHODS

Field Applications

This study was designed as a randomized complete block with 12 replications, where each tree constituted a replicate. Treatments were applied at three timings during the year (summer of 2009, fall of 2009, and winter of 2010). Studies were conducted at two sites; Louisville Colorado and Haigler Nebraska.

The Louisville site (39°58'0.38"N, 105° 8'0.59"W) borders the Dutch Creek Open Space maintained by the city of Louisville, Colorado and is part of the Boulder County Parks and Open Space program. Soils on the site are primarily made up of two soil types; Manter sandy loam with 1 to 3 percent slopes (an Aridic Argiustoll with 15% clay/65.9% sand/ 19.1% silt with 18.9% organic matter, pH 7.2, and a CEC of 17.5) and Ascalon sandy loam with 1 to 3 percent slopes (an Aridic Argiustolls with 10% clay/66.6 % sand/23.4% silt with 2.8% organic matter, pH 7.2, and a CEC of 9.5). The site is a mixture of riparian and upland areas, dominated by large plains cottonwoods (*Populus deltoides* Bartram ex Marsh) and a thick understory of Russian olive trees. During the spring of 2010, contractors working on the site mistakenly treated all of the re-growing stumps with imazapyr. Applications were made after regrowth was visible on the stumps, so data were only collected on the potential for regrowth. No data were collected for subsamples on the number of resprouts or height of the sprouts as had been planned.

The Haigler site (40° 0'27.67"N, 102° 0'29.01"W) is a private cattle ranch near Haigler, Nebraska. The site is bisected by the Arikaree river, with our experiment located on the north side. Tall plains cottonwood trees dominate the riparian area with a thick understory of Russian olive trees that range in size from 3 to 15 inches in trunk diameter. Soils on the site are primarily made up of two soil types; Bankard sand ((an Ustic Torrifluent with 9% clay/82.2% sand/ 8.8% silt) with 0.5% organic matter, pH

8.2, and a CEC of 5) and Bolent-Almeria complex ((an Aquic Ustifluent with 9% clay/82.2 % sand/8.8% silt) with 0.5% organic matter, pH 7.7, and a CEC of 5).

Additional plants on the site include eastern red cedar (*Juniperus virginiana* L. var. *virginiana*), snow on the mountain (*Euphorbia marginata* Pursh), poison ivy (*Toxicodendron radicans* L. Kuntze), barrel cactus (*Echinocereus* Spp. Engelm), prairie june grass (*Koeleria macrantha* Ledeb. Schult), tamarisk (*Tamarix ramosissima* Ledeb) and kochia (*Kochia scoparia* L. Schrad).

At both sites Russian olive trees were cut down using a chainsaw. Once trees were cut, stumps were cut level to create a flat application surface at a minimum of 6 inches above the soil surface. Stump diameters were determined with a tape measure and only Russian olive trees that fell with a 3 to 9 inch diameter size class were used in the experiment. Numbered aluminum tree tags, used for future identification, were later pounded into the pith area of the stumps with careful attention to avoid the cambial layer.

Treatments at both sites included a 100% solution of Bark Oil Blue LT (a petroleum based bark oil), 1% volume to volume (v/v) aminocyclopyrachlor (DPX-MAT 28 SL) mixed with Bark Oil Blue LT, 2.5% v/v mixed with Bark Oil Blue LT, 5% v/v mixed with Bark Oil Blue LT, 30% v/v triclopyr ester (Garlon 4), and a no herbicide/no oil check. Herbicides were applied to the entire cut stump and on the collar to the soil surface at a rate of 1 fl oz of solution per inch of stem diameter. Applications were made with a CO₂ backpack sprayer, calibrated to 45 PSI and applied using a single nozzle boom with a 40-03 E flat fan tip.

Data Collections and Analyses

Visual assessments of control were made 365 days after herbicide/oil applications. Data were collected on % visual regrowth by using a binomial of stump regrowth, with 100 showing no regrowth and 0 representing stumps having any visual regrowth. Data were collected at both sites for regrowth 11 months after treatment, as regrowth was observed before the accidental application of imazapyr at our Louisville site. Subsequent data collections at the Louisville site were not performed.

Binomial percent control data were subject to the following equations:

$$0 = 1/4(n)$$

$$100 = 1 - (1/4(n))^1$$

where n= total sample size. Subsequently, the distribution of these data were checked for normality and then ARCSIN(Sqrt) transformed and subjected to analysis of variance using SAS PROC GLM version 9.2. The interaction of season by treatment was significant and transformed means were separated by Fisher's protected LSD ($\alpha=0.05$), but are presented in their original scale (Table 2.1). Additionally, one-factor contrasts were conducted comparing the three aminocyclopyrachlor treatments to triclopyr ester and to the bark oil control.

Sub sample data for regrowth were collected at the Haigler site only 1 YAT on the number of live stems that had sprouted from treated stumps and the average height (cm) of the resprouted stems. These data were used to calculate a "Regrowth Factor" variable using the equation:

$$RF = [(s/S \times 100) + (h/H \times 100)]$$

Where s = the number of stems per stump, S= the maximum number of stumps recorded for a stump, and h= average height of stems per stump, H= the maximum average height

¹ zumBrunnen, J. 2011. Colorado State University Dept. of Statistics: personal communication

of stems recorded for a stump (Mozdzer et al. 2008). One-way factor contrasts were conducted on regrowth factor data comparing each herbicide treatment to the no herbicide/no oil control for each season and comparing each aminocyclopyrachlor treatment to triclopyr ester for each season.

RESULTS

Visual %-Control

One year after treatment visual evaluations for percent control of Russian olive cut stump applications revealed that there were no differences ($\alpha=0.05$) among our herbicide treatments for all three application timings (Table 2.2). Control ranged from 83 to 100%. Differences were detected; however, among the 100% Bark Oil Blue LT application where the winter application (82%) controlled Russian olive better than the summer (53%) and the fall application (62% control). Differences were also detected among the no herbicide/no oil controls where trees cut down in the summer (29%) controlled Russian olive better than fall (9%) and winter (0%). Additionally, one-factor contrasts showed that the three aminocyclopyrachlor treatments did not differ from the triclopyr ester standard (Table 2.3) but each aminocyclopyrachlor treatment was different than the bark oil control (Table 2.4). These data indicate that all rates of aminocyclopyrachlor were comparable to the industry standard triclopyr ester and adding aminocyclopyrachlor to bark oil increased Russian olive control.

Regrowth Factor

There were no differences for regrowth factor among the 100% basal bark, 1, 2.5, and 5% aminocyclopyrachlor, and the 30% triclopyr ester treatments for the fall and winter applications (Figure 2.2). Differences were detected for regrowth factor for all of the herbicide treatments and the 100% Bark Oil Blue LT compared to the no herbicide/no oil check for both the fall (110%) and the winter (94%) application timings. For the summer application timing regrowth factor, no differences were detected between the four herbicide treatments. The 1% aminocyclopyrachlor (0%), 2.5% aminocyclopyrachlor (0%), 5% aminocyclopyrachlor (0%) and 30% triclopyr ester (0%) allowed no regrowth whereas regrowth factor from the 100% Bark Oil Blue LT (38%). The 1%, 2.5%, 5% aminocyclopyrachlor, 30% triclopyr ester and the 100% Bark Oil Blue LT also allowed less regrowth than the summer check (122%).

DISCUSSION

From our field trials we observed partial Russian olive control 1YAT. Our results correspond with work done by Caplan (2002), who reported that Russian olive trees may reproduce asexually from adventitious shoots developing from roots, in particular, following control applications that do not result in complete death of the trees. During the summer of 2002, Caplan mowed Russian olive trees (if smaller than 8 inches in diameter) or cut down and treated within 5 minutes using a 50% mixture of triclopyr ester (Garlon 4) and water. The following summer, few resprouts were observed from trees less than 8 inches in diameter but the stumps that were larger had a higher proportion of resprouts.

Caplan determined that a single application of the 50% mixture of triclopyr ester and water was inadequate for controlling. Subsequent re-spraying of the foliar regrowth with a 25% mixture of triclopyr ester (Garlon 4) occurred over a 3-year period. Similarly, Edelen and Crowder (1997) performed mechanical removal of the top growth of Russian olive trees in Washington during July and August of 1996. Stumps were treated with two imazapyr rates (either a 2% or a 4% solution). Initial signs of herbicide injury were reported within 3 weeks of application where 75% of the trees receiving the 4% solution had observable damage, whereas only 60% of the trees receiving the 2% solution showed injury.

From a land manager's perspective, acceptable control only occurs when Russian olive trees are completely dead. During our experiment we observed that on occasion stumps that received herbicide applications did resprout. Lateral roots would occasionally produce adventitious shoots (resprouts) within close proximity to the treated stumps as well. Regrowth from the control stumps was much higher (160% RF) compared to an average of 60% RF for the herbicide applications and 79% RF for the 100% basal bark or almost a 10 fold increase in regrowth. Based upon our results from both field sites no herbicide application was completely successful at all three different timings 1 YAT. At particular timings, however, herbicides were 100% effective and killed all Russian olive trees at both of our sites. Our studies indicated that aminocyclopyrachlor was effective at controlling Russian olive during the summer application (both the 1% and 2.5% v/v solutions) and during the fall application with the 5% solution. The standard treatment of a 30% solution of triclopyr ester did not provide complete control of Russian olive trees 1 YAT when applied in the summer and the fall.

Regrowth was not detected at the Haigler site based upon our calculation of regrowth factor, but it was detected at our Louisville site. Triclopyr ester provided 100% control only during the winter application timing for both sites. With the necessity for 100% initial control land managers must choose the appropriate product and timing to provide them with the best control to eliminate the need for treating resprouts.

On an individual stump basis regrowth is highly variable for Russian olive. Pearce and Smith (2001) indicated that Russian olive trees can successfully regenerate when they are in proximity to water. In each of our two field sites seasonal influxes of water were common from flowing streams. Edwards and Beck (2011) found that applications of aminocyclopyrachlor were effective 100% of the time when they were applied on a dryland site. Aminocyclopyrachlor was applied at 2.5%, 5%, 10% and 15% v/v in combination with a petroleum based bark oil (Bark Oil Blue LT) and compared with 30% triclopyr ester, 25% triclopyr ester + 1% imazapyr, 10% aminocyclopyrachlor + 1% imazapyr and a non-treated control. All treatments resulted in 100% visual control of the stumps 1 YAT. According to www.nationalatlas.gov during the period from 1961 through 1990, the Nunn site had an estimated 10.1 to 15 inches of total annual precipitation. Summary information for the CoAgMet weather station located in the town of Ault (8.06 miles from the research site) indicated a total precipitation for all of 2010 at 11.5 inches. In comparison with our seasonal study field sites, the CoAgMet weather station located in the town of Wray, CO (15.06 miles from the research site in Haigler, NE) indicated that the site received approximately 12.8 inches of precipitation in 2010. While there is no apparent difference in precipitation levels, observations on the site by the landowner indicated that the Arikaree river, running through the middle of the

research site, was the highest that it had been in many years, indicating a high level of soil moisture for the area.

Binomial percent control data from our current study for the 2.5% and 5% aminocyclopyrachlor, as well as the 30% triclopyr ester, were compared to the 2009 data from Edwards and Beck (2011) for the 2.5%, 5% aminocyclopyrachlor and the 30% triclopyr ester for the summer timing, as this was the only timing performed in the study (Table 2.5). There were no differences between sites or between treatments; however, this analysis was performed ad hoc, and a future study should be conducted to examine site and seasonal differences between a dryland and a riparian site, performed at the same three timings (e.g. summer, fall and winter) to truly assess these results. While the physiological response of these trees to increased soil moisture has not been researched, this is a future avenue that should be explored to adequately answer the question for land managers who are working in riparian settings and applying herbicides. Future research into the effect of available soil moisture and apparent Russian olive regrowth is warranted. This research is necessary to fully assess the ability of the trees to randomly regrow when treatments to their cut surface are performed in exactly the same manor and with different results.

CONCLUSION

There were no statistical difference among the three application timings (e.g. summer, fall and winter) for aminocyclopyrachlor and the standard triclopyr ester and no differences among the herbicide treatments at any of the timings. However, practical land management deems control as being 100% absolute control. With this standard in

mind, aminocyclopyrachlor was effective for Russian olive control during the summer (1% and 2.5% v/v rates) and fall (5% v/v rate) timings 1 YAT. Triclopyr ester was also highly effective for Russian olive control but, only during the winter application. A “regrowth factor” was only calculated at the Haigler site and was observed sporadically during the fall (1%, 2.5%, 5%) and winter (5%) applications of aminocyclopyrachlor. No regrowth was recorded at the Haigler site for triclopyr ester. It is clear, however, that aminocyclopyrachlor is comparable to the industry standard triclopyr ester to control Russian olive when applied as a cut stump treatment regardless of season of application.

LITERATURE CITED

- Borell, A. E. 1951. Russian olive as a Wildlife Food. *Journal of Wildlife Management* 15: 109-110.
- Bossard, C. 2000. Pages 145–149. in Bossard, C., J. Randall, and M. Hochovsky. eds. *Invasive Plants of California's Wildlands*. Berkeley, CA University of California Press.
- Brock, J.H. 1998. Invasion, Ecology, and Management of *Elaeagnus angustifolia* (Russian Olive) in the Southwestern United States of America. 123-136. In U. Starfinger, K. Edwards, I. Kowarik, and M. Williamson. *Plant Invasions: Ecological Mechanisms and Human Responses*. Backhuys Publishers, Leiden, The Netherlands
- Bukun, B., R.B. Lindenmayer, S.J. Nissen, P. Westra, D.L. Shaner and G. Brunk. 2010. Absorption and Translocation of Aminocyclopyrachlor and Aminocyclopyrachlor Methyl-Ester in Canada Thistle (*Cirsium arvense*). *Weed Science*. 58: 96-102
- Creech, E., and D. Rafferty. 2007. Identification and Management of Russian Olive. University of Nevada Cooperative Extension Fact Sheet 07-39.
- Edelen, W.J., and W.A. Crowder. 1997. Russian olive (*Elaeagnus angustifolia*) Control Experiment Underway (Washington). *Restoration & Management Notes*. 15 (2): 198-199
- Evens, K.E., and D.R. Dietz. 1974. Nutritional energetic of sharp-tailed grouse during winter. *Journal of Wildlife Management*. 38-4:622-629.
- Friedman, J. M., G. T. Auble, P. B. Shafroth, M. L. Scott, M. F. Merigliano, M. D. Freehling, and E. R. Griffin. 2005. Dominance of Non-Native Riparian Trees in Western USA. *Biological Invasions* 7: 747-751.
- Jinks, R.L., and Ciccarese, L. 1997. Effects of Soaking, Washing and Warm Pretreatment on the Germination of Russian olive and Autumn Olive Seeds. *Tree Planters Notes*. Winter/spring 1997: 18-23.
- Katz, G L., and P. B. Shafroth. 2003. Biology, Ecology and Management of *Elaeagnus angustifolia* L. *Wetlands*. 23: 763-777.
- Kindschy, Robert R. 1998. European Starlings Disseminate Viable Russian-Olive Seeds. *Northwestern Naturalist*. 79: 119-20.
- Mozder, T.J., C.J. Hutto, P. A. Clarke, and D.P. Field. 2008. Efficacy of Imazapyr and Glyphosate in the Control of Non-Native *Phragmites australis*. *Restoration Ecology*: June: 221-224.

- Olson, T. E., and F. L. Knopf. 1986. Naturalization of Russian-Olive in the Western United States. *Western journal of applied forestry* 1: 65-69.
- Parker D., and M. Williamson. 2003. Low-Impact, Selective Herbicide Application for Control of Exotic Trees in Riparian Areas: Salt Cedar, Russian olive and Siberian Elm. United States Department of Agriculture: Forests Service-Southwest region.
- Sensenman, S.A. 2007. *Herbicide Handbook* 9th edition. Lawrence, KS: Weed Science Society of America
- Shafroth, P. B., G. T. Auble, and M. L. Scott. 1995. Cottonwood *Populus deltoides* Marshall Subsp. *Monilifera* and Exotic Russian olive *Elaeagnus angustifolia* L. *Conservation Biology* 9: 1169-1175.
- Stannard, M., D. Ogle, L. Holzworth, J Scianna, and E Suleaf. HISTORY, BIOLOGY, ECOLOGY, SUPPRESSION OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA* L.)L.). USDA, NRCS. Boise, ID: USDA, 2002. 1-14.
- Stoleson, Scott H., and Deborah M. Finch. 2001. Breeding Bird Use of and Nesting Success in Exotic Russian olive in New Mexico. *The Wilson Bulletin*. 113: 452-55.
- Tu, M. 2003. Element Stewardship Abstract for *Elaeagnus angustifolia* L. or Russian olive, Oleaster. The Nature Conservancy. Available at:
<http://www.imapinvasives.org/GIST/ESA/esapages/documnts/elaegang.pdf>
- Turner, R.G., J.S. Claus, E. Hidalgo, M.J. Holliday, and G.R. Armel. 2009. Technical introduction of the new DuPont vegetation management herbicide aminocyclopyrachlor. *Weed Science Society of America Abstract*. 49:405.
- Uhing, K. 2008. Russian olive Identification and Management. Colorado Department of Agriculture-Conservation Service division Noxious weed species List B fact sheet.
- Van Dersal, and William R. 1939. Birds that feed on russian olive. *The Auk*. 56: 483-84.
- Zouhar, K. 2005. *Elaeagnus angustifolia*. In: *Fire Effects Information System*, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/>

Table 2.1: ANOVA for Treatments at Both Field Sites

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	30	0.27457696	0.00915257	15.65	<.0001
Error	399	0.23333002	0.00058479		
Corrected Total	429	0.50790698			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	11	0.00626311	0.00056937	0.97	0.4698
Site	1	0.00004660	0.00004660	0.08	0.7779
Trt	5	0.26719059	0.01669941	28.56	<.0001
Season	2	0.00034594	0.00034594	0.59	0.4423
Site*Trt	15	0.03050374	0.00203358	4.24	<.0001
Site*Season	5	0.01626667	0.00325333	6.78	<.0001
Trt*Season	5	0.01970794	0.00394159	7.27	<.0001

Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Summer1% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00120000	0.00120000	2.07	0.1512
Fall 1% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00013333	0.00013333	0.23	0.6320
Winter 1% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00213333	0.00213333	3.68	0.0559
Summer 2.5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00120000	0.00120000	2.07	0.1512
Fall 2.5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00013333	0.00013333	0.23	0.6320
Winter 2.5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00120000	0.00120000	2.07	0.1512
Summer 5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00053333	0.00053333	0.92	0.3383
Fall 5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00120000	0.00120000	2.07	0.1512
Winter 5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00187000	0.00187000	3.21	0.0870
Summer1% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.01613333	0.01613333	27.80	<0.0001
Fall 1% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.00653333	0.00653333	11.26	0.0009
Winter 1% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.00187000	0.00187000	3.21	0.0870
Summer 2.5% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.01613333	0.01613333	27.80	<0.0001
Fall 2.5% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.00653333	0.00653333	11.26	0.0009
Winter 2.5% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.00013333	0.00013333	0.23	0.6320
Summer 5% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.01333333	0.01333333	22.97	<0.0001
Fall 5% v/v MAT 28 v 100% v/v Bark Oil Blue	1	0.01080000	0.01080000	18.61	<0.0001
Winter 5% v/v MAT 28 v 100% v/v Bark Oil Blue LT	1	0.00213333	0.00213333	3.68	0.0559
Summer 30% v/v triclopyr ester v 100% v/v Bark Oil Blue LT	1	0.00853333	0.00853333	14.70	0.0001
Fall 30% v/v triclopyr ester v 100% v/v Bark Oil Blue LT	1	0.00480000	0.00480000	8.27	0.0042
Winter 30% v/v triclopyr ester v 100% v/v Bark Oil Blue LT	1	0.00213333	0.00213333	3.68	0.0559

Table 2.2: Visual %-Control of Russian Olive Using Aminocyclopyrachlor in a Seasonal Cut stump Applications

Treatment ¹	Rate % v/v	Evaluations					
		Summer 2009		Fall 2009		Winter 2010	
		Binomial % control					
Bark Oil Blue LT	100%	54	b	63	b	83	a
Aminocyclopyrachlor ²	1%	100	a	92	a	83	a
Aminocyclopyrachlor	2.5%	100	a	92	a	88	a
Aminocyclopyrachlor	5%	96	a	100	a	92	a
Triclopyr ester ³	30%	88	a	88	a	100	a
Untreated		29	c	8	d	0	d

¹Herbicide treatments tank mixed with Bark Oil Blue LT (petroleum based basal bark oil)

² The aminocyclopyrachlor formulation was DPX-MAT 28 SL (2 lb/gal)

³ The triclopyr ester formulation was a 4 lb/gal

⁴ Means separated by Fishers protected LSD ($\alpha=0.05$). Means followeb by the same letter were not different at the $\alpha=0.05$ level.

Table 2.3: One Factor Contrasts for %-Control of Russian Olive Using Aminocyclopyrachlor and 30% v/v Triclopyr ester in a Seasonal Cut stump Applications

Treatment	Rate	Evaluations		
		Summer	Fall	Winter
		30 % v/v Triclopyr ester		
	% v/v	P value ($\alpha=0.05$)		
Aminocyclopyrachlor ²	1%	0.1512	0.6320	0.0559
Aminocyclopyrachlor	2.5%	0.1512	0.6230	0.1512
Aminocyclopyrachlor	5%	0.3383	0.1512	0.0870

Table 2.4: One Factor Contrasts for %-Control of Russian Olive Using Aminocyclopyrachlor and 100% v/v Bark Oil Blue LT in a Seasonal Cut stump Applications

Treatment	Rate	Evaluations		
		Summer	Fall	Winter
		100 % v/v Bark Oil Blue LT		
	% v/v	P value ($\alpha=0.05$)		
Aminocyclopyrachlor ²	1%	<0.0001	0.0009	0.0870
Aminocyclopyrachlor	2.5%	<0.0001	0.0009	0.6320
Aminocyclopyrachlor	5%	<0.0001	<0.0001	0.0559
Triclopyr ester	30%	0.0001	0.0042	0.0559

Table 2.5: ANOVA for Treatment and Site Between Dryland and Riparian sites

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	3	0.00153654	0.00051218	1.67	0.1788
Error	187	0.02883080	0.00030671		
Corrected Total	215	0.03036735			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Site	1	0.00062374	0.00062374	2.03	0.1572
Trt	2	0.00091568	0.00045784	1.49	0.2300

Chapter 3: Russian Olive Size Effects on Aminocyclopyrachlor Efficacy from Cut Stump Applications

ABSTRACT

Field trials were conducted to evaluate the effect of Russian olive (*Elaeagnus angustifolia* L.) size on aminocyclopyrachlor (DPX-MAT 28 SL) efficacy using cut stump applications. Two size classes of trees were evaluated where trees with 3 to 9 inch diameter trunks comprised one class and those with 9 to 15 inch diameter trunks the other. Trees were cut down and herbicides applied using a CO₂ backpack sprayer with an AA30A MeterJet spray gun (10 ml per trigger pull), applied at 1 fluid oz (30 ml) per 1 inch of trunk diameter to the entire cut surface and root collar. Aminocyclopyrachlor was applied at 1, 2.5, and 5% v/v with JLB oil PLUS and compared to 100% JLB oil PLUS, 30% v/v triclopyr ester with JLB oil PLUS, 2.5% aminocyclopyrachlor with Bark Oil Blue LT, and a no herbicide control. The experiment was 7(herbicide treatments) by 2 (size classes) by 2 (sites) factorial arranged as a RCB with eight replications (one tree per replicate) and conducted at two sites (Hudson, Colorado and Wellington, Colorado). Visual assessment of control was made 1 YAT based on a binomial scale for dead trees (1) and living trees (0). Data were subjected to analysis of variance and means separated by LSD ($\alpha= 0.05$). There were no differences between the two sites so data were combined for analysis. The interaction between treatment and size was significant and reflected in the data where herbicide treatments were more effective for smaller trees.

For the 3 to 9 inch trees herbicide treatments controlled Russian olive similarly (88-100% control) and more effectively than the 100% JLB oil PLUS (50%) and the no herbicide/no oil control trees (47%). For the 9 to 15 inch trees the 1% and 2.5% aminocyclopyrachlor with JLB oil, 30% triclopyr ester with JLB and the 2.5% aminocyclopyrachlor with Bark Oil Blue LT controlled 88 to 93% of Russian olive trees. This was followed by 2.5% aminocyclopyrachlor with JLB (69%), 100% JLB (44%) and the non-treated control trees (5%). Regrowth from the larger trees was highly variable within treatments.

INTRODUCTION

Russian olive (*Elaeagnus angustifolia* L.) is a noxious, perennial tree or a multi-stemmed shrub often found in riparian corridors (Zouhar 2005). Russian olive is a member of the Elaeagnaceae with close relatives in the western United States including silverberry (*Elaeagnus commutate* Bernh. Ex Rydb), buffalo berry (*Shepherdia Canadensis*(L.) Nutt.) and autumn olive (*Elaeagnus umbellate* Thunb) (Katz and Shafroth 2003). Trees can grow up to 40 feet tall and trunks may reach 20 inches in diameter (Stannard et al.. 2002, Zouhar 2005). Trees are saline tolerant, drought tolerant and can form actinorhizal associations with *Frankia* spp., to fix nitrogen (Brock 1998, Olson and Knopf 1986, Stannard et al.. 2002, Zouhar 2005).

Russian olive was introduced from Eurasia sometime in the early 1900's (Shafroth et al.. 1995, Katz and Shafroth 2003, Zouhar 2005). Following its introduction, Russian olive trees were regularly planted in cities, yards, and were subsidized by states and the federal government to be used for hedge plants and windbreaks (Brock 1998, Christensen 1963, Olson and Knopf 1986, Stannard et al.. 2002,). The first evidence of Russian olive trees escaping cultivation were reported in Utah, Nevada, and Arizona between 1941 and 1948 (Christensen 1963, Brock 1998). Within 100 years of its arrival Russian olive has become the fifth most dominant woody riparian species in the western United States (Friedman et al.. 2005).

Cut stump applications of herbicides to the trunk surface are typically highly effective for Russian olive control (Caplan 2002, Creech and Rafferty 2007, Edelen and Crowder 1997, Parker and Williamson 2003, Tu 2003, Zouhar 2005). The combination of physical removal of the top portion of the tree followed by herbicide applications

provides the highest level of control for Russian olive (Parker and Williamson 2003). In cut stump applications, trees are cut down as close to the soil surface as possible using either chainsaws or other forms of mechanical removal (e.g. industrial loppers, etc) and then the cut surface is treated with herbicide. Cuts to the trunk are made horizontal to maximize the surface area and prevent herbicide runoff (Parker and Williamson 2003). Herbicides are applied to the cut surface of the stumps, primarily along the cambial layer, within 5 minutes of cutting to avoid suberization of the exposed tissue and decreased control (Tu 2003, Parker and Williamson 2003). Herbicides that are effective for cut stump applications include triclopyr ester (Garlon 4), triclopyr amine (Garlon 3A), and imazapyr (Habitat or Arsenal) (Creech and Rafferty 2007, Uhing 2008, Zouhar 2005).

Caplan (2002) performed cut stump applications in New Mexico along the Rio Grande River. Russian olive trees were sawed down and treated within 5 minutes with a 50% mixture of triclopyr ester and water. The following summer numerous resprouts were observed from trees that were over 8 inches in diameter. As a result of the observed regrowth researchers concluded that the 50% mixture of triclopyr ester was inadequate for controlling the trees. Subsequent re-spraying of the foliar regrowth with a 25% mixture of triclopyr ester occurred over a 3 year period. Edelen and Crowder (1997) performed mechanical removal of the top growth of Russian olive trees in Washington during July and August of 1996. Stumps were treated with two rates of imazapyr, (either a 2% or a 4% solution) but other application information was not reported. Initial signs of herbicide injury were observed within 3 weeks of application. They reported that 75% of the trees receiving the 4% solution had adequate control, but required future retreatment to provide long lasting control. Similarly, Bossard et al.. (2000)

recommended that applications of 5 to 10 ml of undiluted glyphosate applied immediately to the cambial layer after cutting down provided adequate control during the first year, followed by successive years of monitoring for regrowth and subsequent retreatment.

Aminocyclopyrachlor (6-amino-5chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid or DPX-MAT 28 for the free acid formulation and 6-amino-5chloro-2-cyclopropyl-4-pyrimidine methyl ester DPX-KJM 44 for the methyl ester formulation) is in a new family of chemistry called the pyrimidine carboxylic acids under the synthetic-auxic herbicides mode of action (Bukun et al.2008, Sensenman 2007). Structurally, aminocyclopyrachlor is very similar to the pyridine carboxylic herbicides, such as picloram, (Tordon 22K), aminopyralid, (Milestone) and clopyralid (Transline) (Sensenman 2007). However, the aminocyclopyrachlor molecule differs from these in that it possesses additional nitrogen in its heterocyclic carbon ring structure and a cyclopropyl side chain (Bukun et al. 2010).

Field trials have been conducted on aminocyclopyrachlor efficacy by private industry and university weed scientists (Bukun et al. 2010). The aminocyclopyrachlor molecule has shown promise in the control of many noxious and invasive weeds (E.I. DuPont, 2009) and is being targeted for use on rangelands, pastures, forestry, non-cropland areas, right of ways, industrial areas and natural areas (Bukun et al. 2010, Sensenman 2007, Turner et al.. 2009).

The objective of our research was to investigate the effects of Russian olive tree size on efficacy of cut stump application with aminocyclopyrachlor and determine if control was comparable to the industry standard triclopyr ester. The first null hypothesis

for these experiments would be Ho1: the relative trunk size of Russian olive will not influence the efficacy among herbicides applied as a cut stump treatments. The first alternate hypothesis for these experiments would be Ha1: the relative trunk size of Russian olive trees will influence the efficacy among herbicides applied as a cut stump treatment. The second null hypothesis would be Ho2: control of Russian olive from aminocyclopyrachlor will not be comparable to the industry standard triclopyr ester applied as cut stump treatments. The second alternate hypothesis would be Ha2: control of Russian olive from aminocyclopyrachlor will be comparable to the industry standard triclopyr ester applied as cut stump treatments.

MATERIALS AND METHODS

Field Applications

Herbicides were applied to Russian olive trees at two sites; the Wellington Number 4 reservoir near Wellington, Colorado and the Banner Lakes State Wildlife refuge near Hudson, Colorado. The study was designed as a 7 (herbicide treatments) by 2 (size classes) by 2 (sites) factorial arranged as a randomized complete block with eight replications, where a single tree constituted a replicate.

The Wellington Number 4 Reservoir (40°43'8.89"N by 105° 1'46.54"W) is a lake managed by the Poudre Valley Irrigation Company and the Colorado Division of Wildlife and is located near Wellington, CO. Soils at the site are primarily Cushman fine sandy loams (an Ustic Haplargids with 15% clay, 65.4% sand, 19.6% silt with 1.5% organic matter, pH 7.2 and a CEC of 10 (NRCS Web Soil Survey²). The study site is located on the north shore of the lake, which is not accessible to the public. Russian olive trees on

² USDA-NRCS web soil survey available at <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>

the site are a mixture of older trees (roughly 9 to 25 inches in diameter) with smaller stands of medium trees (about 4 to 8 inches in diameter). The lake shore is dominated by smaller trees (roughly 2 to 4 inches in diameter) as a result of the lake level being lowered for irrigation. Additional vegetation on the site includes thick stands of plains cottonwood (*Populus deltoides* Bartram ex Marsh. ssp. *monilifera* Aiton Eckenwalder) that dominate both the north and south shores of the lake. However, cottonwood trees are beginning to die off from possible competition for water from the increased number of Russian olive trees that have sprouted underneath them.

The Banner Lakes site (40° 5'14.14"N by 104°33'35.36"W), near Hudson, CO is a part of the Colorado Division of Wildlife's system of managed properties and serves as a state wildlife refuge for many migratory birds and waterfowl. Soils in the area include Colby loams on a 1-3% slope (an Aridic Ustorthents with 21% clay, 36.9% sand, 42.1% silt with 1.25% organic matter, pH 7.9 and a CEC of 12.5) and Colby-Adena loams on a 3 to 9% slope (Aridic Ustorthents with 21% clay, 36.9% sand, 42.1% silt with 1.25% organic matter, pH 7.9 and a CEC of 12.5) (NRCS³ Web Soil Survey). The Russian olive trees were planted by the Colorado Division of Wildlife to provide a windbreak and some level of structural diversity, which is absent across the site, except in remote areas near marshes. Since their plantings, the Russian olive trees on the site have spread to cover many of the lake shores of nine surrounding ponds. The Colorado Division of Wildlife views the trees as a massive problem and has engaged in removal operations using cut stump applications with either triclopyr ester or imazapyr.

In our experiment, Russian olive trees were cut down with a chainsaw. Once trees were cut, stumps were cut a second time perpendicular to the ground to create a flat

³ USDA-NRCS web soil survey available at <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>

application surface, at a minimum of 6 inches above the soil surface and then stump diameter was measured. Only Russian olive trees that fell into our two size classes (3 to 9 inches of trunk diameter or 9 to 15 inches of trunk diameter) were used for the experiment.

Treatments included a 100% solution of JLB oil PLUS (a vegetable oil based bark oil that has been blended with a blue dye to track applications), 1% v/v aminocyclopyrachlor (DPX-MAT 28 SL) mixed with JLB oil PLUS, 2.5% v/v aminocyclopyrachlor mixed with JLB oil PLUS, 5% v/v aminocyclopyrachlor mixed with JLB oil PLUS and compared to 30% v/v triclopyr ester (Garlon 4) mixed with JLB oil PLUS, 2.5% v/v aminocyclopyrachlor mixed with Bark Oil Blue LT (a petroleum based basal bark oil mixed with a blue dye to track applications), and a no herbicide/no oil control.

Herbicides were applied to the entire cut surface and root collar at an application rate of 1 fluid oz of solution per inch of trunk diameter. Applications were made with a CO₂ backpack sprayer calibrated to 45 PSI and applied using an AA30A MeterJet spray gun and calibrated to apply exactly 10 ml of solution in a single trigger pull. Numbered aluminum tree tags were pounded into the pith area of the stumps with careful attention to avoid the cambial layer following applications.

Data Collection and Analysis

Visual evaluations of control were made approximately 11 months after herbicide applications. Data were collected on % visual regrowth using a binomial system to represent stump regrowth, with 100 being no visual sign of regrowth and 0 representing

stumps with any visual signs of regrowth. Binomial percent control data were subjected to the equations:

$$0=1/4(n)$$

$$100= 1-(1/4(n))^4$$

Where n= the total sample size. Subsequently these data were checked for normality and transformed using ARCSIN(Sqrt) and transformed data then subjected to analysis of variance using SAS version 9.2 by a PROC GLM procedure (Table 3.1). A treatment by size interaction was detected and the transformed data were separated by a Fisher's protected LSD ($\alpha=0.05$) but are presented in their original scale. One-factor contrasts were conducted (Table 3.1) comparing each aminocyclopyrachlor rate to the industry standard triclopyr ester for each size class; compared each aminocyclopyrachlor rate and triclopyr ester to the JLB oil control; and compared the 2.5% aminocyclopyrachlor rate mixed with JLB oil to the same aminocyclopyrachlor rate mixed with bark oil blue.

RESULTS

All herbicide treatments controlled the 3 to 9 inch trees similarly (88 to 100% control; Table 3.1). All the herbicide treatments were more effective at controlling trees than the 100% JLB oil PLUS (50%) and the no herbicide/no oil control (47%).

Aminocyclopyrachlor at 1 and 5% v/v with JLB oil PLUS and 30% triclopyr with JLB oil PLUS controlled 93% of Russian olive trees 9 to 15 inches in diameter 1 YAT.

Aminocyclopyrachlor at 2.5% with JLB oil PLUS controlled only 69% of large Russian olive trees and the same herbicide rate PLUS the Bark Oil Blue carrier controlled 88% of large Russian olive trees and these treatments were statistically similar. The 100% JLB

⁴ zumBrunnen, J. 2011. Colorado State University Dept. of Statistics: personal communication

controlled fewer large trees (44%) but still more than attrition of large non-treated control trees (5%). One-factor contrasts comparing herbicide treatments within the small size class showed all rates of aminocyclopyrachlor controlled Russian olive similarly to triclopyr ester (Table 3.3). When herbicide treatments were compared within the large size class all rates of aminocyclopyrachlor controlled Russian olive comparably to triclopyr ester except the 2.5% rate (Table 3.3). One-factor contrasts comparing the JLB oil PLUS control herbicides revealed that all rates of aminocyclopyrachlor and triclopyr ester mixed with JLB oil PLUS provided superior control (Table 3.4). One-factor contrasts also revealed no difference between 2.5% aminocyclopyrachlor mixed with JLB oil PLUS or Bark Oil Blue (Table 3.5).

DISCUSSION

The question of tree size influencing control for cut stump applications of Russian olive trees has been raised in several studies. In Caplan's (2009) study, trees were either mowed (if smaller than 8 inches in diameter) or cut down with chainsaws (if larger than 8 inches in diameter). All trees in the study were treated with a 50% triclopyr ester and water solution within 5 minutes of cutting. The following year Caplan observed that trees smaller than 8 inches had few re-sprouts, while larger trees had a higher occurrence of resprouting. Edwards et al. (2009) conducted a study in 2007 to evaluate the effects of aminocyclopyrachlor (DPX-KJM 44) applied to Russian olive trees as a foliar application. Trees were sprayed with a hand held AA43 Gun Jet spray gun, with a D8 orifice disk in a spray-to-wet application. Treatments consisted of 2, 4, 6, and 8 oz ai/A of DPX-KJM 44 compared to 2 oz ai/A DPX-KJM 44 mixed with 1% v/v Dyne-amic

surfactant, 2 and 4 oz ai/A DPX-MAT 28, 7 oz ai/A imazapyr, 2 oz ai/A metsulfuron, and untreated controls. Trees were separated into two size classes (shorter than 8 feet tall and taller than 8 feet tall). Defoliation data were collected 1 YAT. The results indicated that 90 to 100% of Russian olive trees were controlled at 8 oz ai/A rate and control progressively decreased as rate decreased. The data also suggested that larger trees were less susceptible to herbicides and trees less than 10 feet tall were more susceptible to the herbicide because trees taller than 10 feet regrew and developed new foliage.

Our results indicate that there were no statistical differences in control of Russian olive trees based upon relative size of the trunk, except for the 2.5% v/v aminocyclopyrachlor + JLB oil PLUS application. However, from a land managers perspective, any control less than 100% 1 YAT is considered unsuccessful. No herbicide treatments applied to larger trees (9 to 15 inches) resulted in 100% control. For the smaller trees (3 to 9 inches), 100 % control was achieved for the 1% and 2.5% aminocyclopyrachlor + JLB oil PLUS, 30% triclopyr standard and the 2.5% aminocyclopyrachlor+ Bark Oil Blue LT. These results directly reflect both Caplan (2009) and Edwards' (2009) studies in which larger trees appear not to be as susceptible to herbicide applications as smaller trees.

Within their respective size classes, herbicide treatments were different from the control populations and from the 100% JLB oil PLUS (Table 3.2). For both sizes there was an increase in control from the 100% JLB oil PLUS (average of 44% control) to the herbicide treatments (average of 93% control), indicating that it is necessary to include a herbicide in any cut stump application to achieve adequate control. These results confirm earlier findings of Edwards and Beck (2011) that cut stump applications using only the

100% Bark Oil Blue LT treatment had statistically higher levels of regrowth compared to treatments including herbicides. The inclusion of the herbicide with the phytotoxic properties of the basal oils, appear to boost control of Russian olive trees 1 YAT. For land managers, these findings are important for designing future cut stump applications to reduce the level of regrowth and indirectly the numbers of trees and out of pocket costs that contractors face to retreat the following year.

All cut stump rates of aminocyclopyrachlor applied to 3 to 9 inch diameter trees controlled Russian olive similarly to a standard 30% triclopyr ester treatment where 88 to 100% were controlled 1 YAT. One-factor contrasts showed the same effect. We compared two oil carriers to determine if efficacy would be influenced and found that 100% of small Russian olive trees were controlled whether the 2.5% v/v aminocyclopyrachlor was mixed with Bark Oil Blue LT (for terrestrial systems) and JLB Oil PLUS (for riparian systems). Our results indicate that 1% aminocyclopyrachlor or 30% triclopyr ester mixed with JLB oil PLUS applied as cut stump would adequately control Russian olive trees 9 inches in diameter or smaller.

For the 9 to 15 inch size class, no herbicide application controlled Russian olive trees 100% of the time 1 YAT. Aminocyclopyrachlor at 1 and 5% with JLB Oil PLUS were similar to a standard 30% triclopyr ester, controlling 93 to 94% of Russian olive 1 YAT. Additionally, one-factor contrasts showed a similar effect. However, all of these applications would be unacceptable to land managers who are trying to limit the need for costly retreatment for regrowing stumps because no treatment provided 100% initial control. Land managers must therefore keep the size of the trees they are treating in mind

when they conduct cut stump applications to account for larger trees having a higher probability for regrowth the following growing season.

CONCLUSION

Our research indicates that all rates of aminocyclopyrachlor controlled Russian olive similarly to the industry standard triclopyr ester within the 3 to 9 inch size class and all but the 2.5% rate within the 9 to 15 inch size class. Our research thus demonstrated that there was a difference in control between Russian olive trees depending upon the relative trunk size. For trees 3 to 9 inches in diameter applications of 1 or 2.5% aminocyclopyrachlor mixed with JLB oil, 30% triclopyr ester with JLB oil or 2.5% aminocyclopyrachlor mixed with Bark Oil Blue LT offered excellent (100%) control of trees 1 YAT. For trees 9 to 15 inches in diameter no herbicide offered 100% control of stumps 1 YAT. From a land managers perspective, treatments to Russian olive trees larger than 9 inches in diameter will possibly require retreatment the following growing season.

LITERATURE CITED

- Bossard, C. 2000. Pages 145–149. in Bossard, C., J. Randall, and M. Hochovsky. eds. Invasive Plants of California's Wildlands. Berkeley, CA University of California Press.
- Brock, J.H. 1998. Invasion, Ecology, and Management of *Elaeagnus angustifolia* (Russian olive) in the Southwestern United States of America. 123-136. In U. Starfinger, K. Edwards, I. Kowarik, and M. Williamson. Plant Invasions: Ecological Mechanisms and Human Responses. Backhuys Publishers, Leiden, The Netherlands
- Bukun, B., R. B. Lindenmayer, S. J. Nissen, P. Westra, D. L. Shaner and G. Brunk. 2010. Absorption and translocation of aminocyclopyrachlor and aminocyclopyrachlor methyl-ester in Canada Thistle (*Cirsium arvense*). Weed Sci. 58:96-102
- Caplan, T. 2002. Controlling Russian olive within Cottonwood Gallery Forests along the Middle Rio Grande Floodplain (New Mexico). Ecological Restoration. 20 (2):138-139
- Christensen, E. M. 1963. Naturalization of Russian olive (*Elaeagnus angustifolia* L.) in Utah. American Midland Naturalist. 70-1:133-137.
- Creech, E., and D. Rafferty. 2007. Identification and Management of Russian olive. University of Nevada Cooperative Extension Fact Sheet 07-39.
- Edelen, W.J., and W.A. Crowder. 1997. Russian olive (*Elaeagnus angustifolia*) control experiment underway (Washington). Restoration & Management Notes. 15 (2):198-199.
- Edwards, R. J., J. Sebastian, and K. G. Beck. 2009. Variability of Russian olive control using herbicides. Abstract 7 in Proceedings of the 62nd annual WSSA meeting in Albuquerque, NM.
- Edwards, R.J., and K.G. Beck. 2011. Control of Russian Olive Through Cut Stump and Basal Bark Herbicide Applications. Abstract 138 in Proceedings of the 62nd annual WSWS meeting in Albuquerque, NM.
- E.I. DuPont de Nemours and Company. 2009. DuPont DPX-MAT 28 Herbicide Technical Bulletin
- Friedman, J. M., G. T. Auble, P. B. Shafroth, M. L. Scott, M. F. Merigliano, M. D. Freehling, and E. R. Griffin. 2005. Dominance of Non-Native Riparian Trees in Western USA. Biological Invasions 7: 747-751.
- Katz, G L., and P. B. Shafroth. 2003. Biology, Ecology and Management of *Elaeagnus angustifolia* L. Wetlands. 23: 763-777.

- Olson, T. E., and F. L. Knopf. 1986a. Naturalization of Russian-Olive in the Western United States. *Western journal of applied forestry* 1: 65-69.
- Parker D., and M. Williamson. 2003. Low-Impact, Selective Herbicide Application for Control of Exotic Trees in Riparian Areas: Salt Cedar, Russian olive and Siberian Elm. United States Department of Agriculture: Forests Service-Southwest region.
- Sensenman, S.A. 2007. *Herbicide Handbook* 9th edition. Lawrence, KS: Weed Science Society of America
- Shafroth, P.B, G.T. Auble, and M.L.Scott. 1995. Germination and establishment of native plains cottonwood (*Populus deltoides* marshall subsp. *monilifera*) and exotic russian-olive (*Elaeagnus angustifolia* L.). *Conservation Biology*. 9: 1169-1175.
- Stannard, M., D. Ogle, L. Holzworth, J Scianna, and E Suleaf. HISTORY, BIOLOGY, ECOLOGY, SUPPRESSION OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA* L.)L.)L.). USDA, NRCS. Boise, ID: USDA, 2002. 1-14.
- Tu, M. 2003. Element Stewardship Abstract for *Elaeagnus angustifolia* L. or Russian olive, Oleaster. The Nature Conservancy. Available at:
<http://www.imapinvasives.org/GIST/ESA/esapages/documnts/elaeang.pdf>
- Turner, R.G., J.S. Claus, E. Hidalgo, M.J. Holliday, and G.R. Armel. 2009. Technical introduction of the new DuPont vegetation management herbicide aminocyclopyrachlor. *Weed Science Society of America Abstract*. 49:405.
- Uhing, K. 2008. Russian olive Identification and Management. Colorado Department of Agriculture-Conservation Service division Noxious weed species List B fact sheet.
- Zouhar, K. 2005. *Elaeagnus angustifolia*. In: *Fire Effects Information System*, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/>

Table 3.1: ANOVA for treatments at both field sites

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	21	0.12583530	0.00599216	9.29	<.0001
Error	200	0.12901155	0.00064506		
Corrected Total	221	0.25484685			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	7	0.00622969	0.00088996	1.38	0.2156
Site	1	0.00964228	0.00964228	14.95	0.0001
Trt	6	0.09266816	0.01544469	23.94	<0.0001
Size	1	0.00766695	0.00766695	11.89	0.0007
Trt*Size	6	0.00887159	0.00147860	2.29	0.0367
Trt*Site	6	0.02462069	0.00410345	7.79	<0.0001
Size*Site	1	0.00293432	0.00293432	5.57	0.0193

Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
3-9 in 1% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00760000	0.00760000	5.65	0.0670
3-9 in 2.5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00650000	0.00650000	6.98	0.0780
3-9 in 5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00650000	0.00650000	6.98	0.0780
9-15 in 1% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00320000	0.00320000	4.64	0.0324
9-15 in 2.5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00080000	0.00080000	1.16	0.2827
9-15 in 5% v/v MAT 28 v 30% v/v triclopyr ester	1	0.00074400	0.00074400	5.54	0.0650
3-9 in 1% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
3-9 in 2.5% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
3-9 in 5% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
3-9 in 30% v/v triclopyr ester v 100% v/v JLB oil PLUS	1	0.00320000	0.00320000	4.64	0.0324
9-15 in 1% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.00720000	0.00720000	10.44	0.0014
9-15 in 2.5% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
9-15 in 5% v/v MAT 28 v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
9-15 in 30% v/v triclopyr ester v 100% v/v JLB oil PLUS	1	0.01280000	0.01280000	18.56	<0.0001
3-9 in 2.5% v/v MAT 28 (JLB)v 2.5% v/v MAT 28 (BOB)	1	0.00072000	0.00072000	0.54	0.3457
9-15 in 2.5% v/v MAT 28 v 2.5% v/v MAT 28 (BOB)	1	0.00180000	0.00180000	2.61	0.1077

Table 3.2: Visual %-Control of Russian Olive Trunk Size Effects Following Cut Stump Applications

Treatment	Rate	Evaluations			
		<u>2011</u>		<u>2011</u>	
		3-9 inch trunks	Binomial % control	9-15 inch trunks	Binomial % control
JLB oil PLUS ¹	100%	50	cd	44	d
Aminocyclopyrachlor ^{2*}	1%	100	a	94	a
Aminocyclopyrachlor*	2.5%	100	a	69	bc
Aminocyclopyrachlor*	5%	88	ab	94	a
Triclopyr ester ^{3*}	30%	100	a	93	a
Aminocyclopyrachlor +Bark Oil Blue LT ⁴	2.5%	100	a	88	ab
Untreated		47	cd	6	e

¹ JLB oil PLUS (a vegetable based basal bark oil)

*Herbicide treatments tank mixed with JLB oil PLUS

² The aminocyclopyrachlor formulation was DPX-MAT 28 SL (2 lb/gal)

³ The triclopyr ester formulation was a 4 lb/gal

⁴ Bark Oil Blue LT (a petroleum based basal bark oil)

⁵ Means separated by a Fishers protected LSD ($\alpha=0.05$). Means followeb by the same letter were not different at the $\alpha=0.05$ level.

Table 3.3: One Factor Contrasts for %-Control of Russian Olive Trunk Size Effects Following Cut Stump Applications Using Aminocyclopyrachlor compared to 30% v/v Triclopyr ester

Treatment	Rate	Evaluations	
		30 % v/v Triclopyr ester	
		P value ($\alpha=0.05$)	
	% v/v	3-9 inch	9-15 inch
Aminocyclopyrachlor ²	1%	0.0670	0.0780
Aminocyclopyrachlor	2.5%	0.0780	0.0324
Aminocyclopyrachlor	5%	0.2827	0.0650

Table 3.4: One Factor Contrasts for %-Control of Russian Olive Trunk Size Effects Following Cut Stump Applications Using Aminocyclopyrachlor and 30% v/v Triclopyr ester compared to 100% v/v JLB oil PLUS

Treatment	Rate	Evaluations	
		30 % v/v Triclopyr ester	
		P value ($\alpha=0.05$)	
	% v/v	3-9 inch	9-15 inch
Aminocyclopyrachlor ²	1%	<0.0001	<0.0001
Aminocyclopyrachlor	2.5%	<0.0001	0.0324
Aminocyclopyrachlor	5%	0.0014	<0.0001
Triclopyr ester	30%	<0.0001	<0.0001

Table 3.5: One Factor Contrasts for %-Control of Russian Olive Trunk Size Effects Following Cut Stump Applications Using 2.5% v/v Aminocyclopyrachlor + JLB oil PLUS and 2.5% v/v Aminocyclopyrachlor + Bark Oil Blue LT

Treatment	Rate	Evaluations	
		2.5% v/v Aminocyclopyrachlor + Bark Oil Blue LT	
		P value ($\alpha=0.05$)	
		3-9 inch	9-15 inch
Aminocyclopyrachlor + JLB oil PLUS	2.5%	0.3457	0.1077

Chapter 4: Using Aminocyclopyrachlor as a Hack and Squirt Application to Control Russian Olive

ABSTRACT

Field trials were conducted on Russian olive (*Elaeagnus angustifolia* L.) trees testing aminocyclopyrachlor (DPX-MAT 28 SL) efficacy for hack and squirt applications. Trees were hacked with a hand held hatchet at a rate of one hack per 3 inches of trunk diameter and 1 ml of herbicide was applied per hack using a syringe. Treatments included aminocyclopyrachlor, imazapyr (Habitat), glyphosate (Rodeo), aminopyralid (Milestone), triclopyr amine (Garlon 3A), Milestone VM+ (10:1 triclopyr amine + aminopyralid), and a 50:50 aminocyclopyrachlor + triclopyr amine mixture. The experiment was designed as an 8(treatments) by 2 (sites) in a factorial design arranged as a RCB with eight replications (1 tree per replicate) and conducted at two sites to be (Nunn, Colorado and Wellington, Colorado). Visual assessments of control were made 1 year after treatment (YAT) based on a 0 to 100% visual percent control scale for necrosis. Data were transformed to a log scale and subjected to analysis of variance and means separated by LSD ($\alpha= 0.05$). We concluded that aminocyclopyrachlor was an effective herbicide for use in hack and squirt applications achieving 91% control of Russian olive

trees 1 YAT. This level of control was comparable to both industry standards glyphosate and imazapyr (94% and 98%, respectively). A 50:50 mixture of aminocyclopyrachlor and triclopyr amine resulted in 98% control of Russianolive trees 1YAT. Aminopyralid containing products (e.g. Milestone and Milestone VM+) offered less percent control (84% and 89%, respectively), than aminocyclopyrachlor. Triclopyr amine had the lowest percent control when applied alone at both field sites (77%).

INTRODUCTION

Russian olive is a noxious perennial tree or a multi-stemmed shrub, most commonly found in habitats ranging from moist riparian corridors, to prairies (both tallgrass and shortgrass), and to dry deserts (Zouhar 2005). Trees are easily identified by their pale green leaves, reddish bark and sharp thorns along the branches (Zouhar 2005). Trees exceed heights of 40 feet and trunks may reach 20 inches in diameter (Stannard et al., 2002, Zouhar 2005). Russian olive trees are saline tolerant, drought tolerant, and form actinorhizal associations with *Frankia* spp., to fix nitrogen (Brock 1998, Olson and Knopf 1986, Stannard et al., 2002, Zouhar 2005).

Frill or “hack and squirt” applications have been an effective tool for land managers to eliminate trees (Parker and Williamson 2003, Dieter 2000). Hack and squirt applications are considered the precursor to the cut stump and the basal bark applications. These applications involve a two part process, including a “hack”, or a direct cut into cambial layer of a tree, and a “squirt” of a particular herbicide into the wound. This injection is thought to minimize the overall amount of herbicide needed to kill trees and represents a direct application to a particular target instead of a broadcast application. The hack and squirt technique is thought to maintain nesting and cover for many avian species, by limiting collateral habitat damage caused by more aggressive techniques, such as cut stump (complete tree removal) and foliar applications (Clubine 2008).

A hand held hatchet is used to make a “hack” into the cambium layer of selected trees, creating a 1 to 1.25 inch “cup” in which undiluted herbicides can be injected (Clubine 2008). Hack and squirt treatments can also be performed with a tool called a hypo-hatchet, in which herbicides are injected into a tree simultaneously as hacks are

made. This hack is meant to create a direct avenue into the phloem tissue of the plant to increase transport of herbicides to meristems (Parker and Williamson 2003). Moorhead (2003) recommends that hack and squirt applications be made using a technique that calls for one hack for every 3 inches of trunk diameter with 1 ml of herbicide solution applied to each cut.

Previous research into the hack and squirt technique indicates excellent control of mature Russian olive trees (Tu 2003). Stannard et al.(2002) reported that both imazapyr and glyphosate can be applied undiluted into frill cuts and provide excellent control of trees. Glyphosate has been shown to be highly effective during winter applications (Stannard et al.2002). Dieter (2000) recommended that applications be made directly into the cambial layer should be made as close to the ground as possible and herbicides should be directly applied to these frill cuts. Parker and Williamson (2003) report that 50% mixtures of triclopyr amine (Garlon 3A) in water and mixtures of 50% triclopyr amine (Garlon 3A) + 3 oz of imazapyr (Habitat or Arsenal) in water offer good control of Russian olives using this technique.

Field trials are now being conducted on DuPont Crop Protection's new chemistry aminocyclopyrachlor (6-amino-5chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid or DPX-MAT 28 for the free acid formulation and 6-amino-5chloro-2-cyclopropyl-4-pyrimidine methyl ester for DPX-KJM 44, the methyl ester formulation) by private industry and university weed scientists (Bukun et al. 2010). The aminocyclopyrachlor molecule has shown promise in the control of many noxious and invasive weeds (E.I. DuPont, 2009). Aminocyclopyrachlor is being targeted for applications on rangelands, pastures, forestry, non-cropland areas, rights-of -way, industrial areas, and natural areas

(Bukun et al. 2010, Sensenman 2007, Turner et al... 2009). Aminocyclopyrachlor (DPX-MAT 28) is in a new family of chemistry called the pyrimidine carboxylic acids under the synthetic-auxic herbicides mode of action (Bukun et al.. 2008, Sensenman 2007). Structurally, aminocyclopyrachlor is very similar to the pyridine carboxylic herbicides, such as picloram, (Tordon 22K), aminopyralid, (Milestone) and clopyralid (Transline) (Sensenman 2007). However, the aminocyclopyrachlor molecule differs from these in that it possesses additional nitrogen in its heterocyclic carbon ring structure and a cyclopropal side chain (Bukun et al.2010).

The purpose of this research was, two-fold; 1) Evaluate the efficacy of aminocyclopyrachlor as a potential herbicide for hack and squirt applications to Russian olive trees and 2) Compare aminocyclopyrachlor to known industry standards currently labeled for hack and squirt applications: (imazapyr (Habitat), glyphosate(Rodeo), Aminopyralid (Milestone), triclopyr amine (Garlon 3A), Milestone VM+ (10:1 triclopyr amine + aminopyralid), and a 50:50 aminocyclopyrachlor + triclopyr amine mixture. The null hypotheses for these experiments are Ho1: aminocyclopyrachlor will not be suitable to use as a hack and squirt technique to control Russian olive; Ho2: aminocyclopyrachlor will not control Russian olive via a hack and squirt methodology as well and current industry standards. The alternate hypotheses for these experiments are Ha1: aminocyclopyrachlor will be a suitable herbicide to control Russian olive by the hack and squirt technique; Ha2: aminocyclopyrachlor will control Russian olive more effectively by the hack and squirt technique than currently used industry standards

MATERIALS AND METHODS

Field Applications

The experiment was designed as an 8 (treatments) by 2 (sites) factorial arranged as a RCB with eight replications (1 tree per replicate) and conducted at two sites (Nunn, Colorado and Wellington, Colorado) in July 2010. Treatments included aminocyclopyrachlor (DPX-MAT 28 SL), aminopyralid (Milestone), aminopyralid + triclopyr (Milestone VM+), glyphosate (Rodeo), imazapyr (Habitat), triclopyr amine (Garlon 3A), and a 50:50 mixture of aminocyclopyrachlor and triclopyr amine. Treatments were applied at two sites; Nunn, Colorado and the Wellington Number 4 reservoir near Wellington, Colorado.

The Nunn site (40°44'50.11"N by 104°46'23.48"W) is a former pasture area with the Lone Tree Creek running through it, however the stream has been dry for several years. The site became dominated by Russian olive trees following a flood in the early 1980s. The area is now a mixture of older trees (roughly 10 to 14 inches in trunk diameter) and several new groves of smaller trees (3 to 8 inches in trunk diameter). Soils in the area are primarily dominated by Haverson loam (an Aridic Ustifluvents with 18.5% clay, 43% sand, 38.5% silt with an organic matter of 1.25%, pH 7.9 and a CEC of 12.5 (NRCS Web Soil Survey⁵). The area is dotted with plains cottonwood (*Populus deltoides* Bartram ex Marsh) along with the Russian olive and the understory is dominated primarily by smooth brome (*Bromus inermis* Leyss), Canada thistle (*Cirsium arvense* L. Scop.), kochia (*Kochia scoparia* L. Schrad), prickly pear cactus (*Opuntia polyacantha* Haw.), tumble mustard (*Sisymbrium altissimum* L.), and downy brome (*Bromus tectorum* L.).

⁵ USDA-NRCS web soil survey available at <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>

The Wellington Number 4 Reservoir (40°43'8.89"N by 105° 1'46.54"W) is a lake managed by the Poudre Valley Irrigation Company and the Colorado Division of Wildlife. Soils around the lake are primarily Cushman fine sandy loams (an Ustic Haplargids with 15% clay, 65.4% sand, 19.6% silt with 1.5% organic matter, pH 7.2 and a CEC of 10 (NRCS Web Soil Survey⁶). The study site is located on the north shore of the lake, which is not accessible to the public. Russian olives on the site are a mixture of older trees (roughly 9 to 25 inches in trunk diameter) with smaller stands of medium trees (roughly 4 to 8 inches in trunk diameter). The old lake shore is dominated by smaller Russian olive trees (roughly 2 to 4 inches in trunk diameter) as a result of the lake level being lowered for irrigation. Thick stands of plains cottonwood dominate both the north and south shores of the lake, but appear to be dying out from the increased competition from the large stands of Russian olive that have developed beneath them.

Trees selected for this study had a single trunk and fell into a 3 to 9 inch trunk size class. Lower branches were removed to provide access to the trunk. Trunk diameters were estimated by measuring the circumference 1 ft above the soil surface. Using a hatchet, one hack was made every 3 inches around the circumference of the trunk of the tree. Hacks were performed at least 1 ft above of the soil surface at a 45° angle to the ground and into the cambium layer of the trees. Each frill provided a small reservoir at the bottom that was free of wood chips. Using a 10 ml syringe 1 ml of herbicide was injected into the frill at the base of the reservoir. The 50:50 mixture of aminocyclopyrachlor and triclopyr amine had to be applied separately (1/2 ml of aminocyclopyrachlor followed by 1/2 ml of triclopyr amine) as tank mixing the two compounds resulted in incompatibility of the herbicides. All herbicides were placed into

⁶ USDA-NRCS web soil survey available at <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>

the frill slowly to prevent spillage, seepage and splash back of the liquids out of the frill. Numbered aluminum tree tags were pounded into the trunks of the trees for later identification.

Data Collections and Analysis

Visual evaluations of control were conducted approximately 1 year after treatment (1YAT) based on a percent defoliation on a 0 to 100% scale, with 0 being no defoliation and 100% being complete defoliation. Percent control data were log transformed and subjected to analysis of variance using SAS version 9.2 using a PROC GLM procedure (Table 4.1). Means for treatment were significant and were separated by a Fisher's protected LSD ($\alpha=0.05$) (Figure 4.2). While data were analyzed as log transformations, data are presented in their original scale (Figure 4.2). One-factor contrasts were conducted (Table 4.1) comparing aminocyclopyrachlor to the other herbicides used in our study; and compared the 50:50 mixture of aminocyclopyrachlor + triclopyr ester to the other herbicides used in our study.

RESULTS

Control of Russian olive trees 1 year after hack and squirt applications varied from 77% to 98%, with a 50:50 mixture of aminocyclopyrachlor + triclopyr amine and imazapyr offering the best control. Glyphosate achieved 94% control. When applied separately, aminocyclopyrachlor provided 91%, triclopyr ester only 77% and aminopyralid 84% control of Russian olive trees 1 YAT. The other aminopyralid containing product, Milestone VM+ (10:1 mixture of triclopyr + aminopyralid) provided

89% control 1 YAT. One-factor contrasts comparing aminocyclopyrachlor treatment to other herbicides used in our hack and squirt experiment showed aminocyclopyrachlor controlled Russian olive similarly to a 50:50 solution of aminocyclopyrachlor + triclopyr ester, glyphosate and imazapyr. One-factor contrasts comparing the 50:50 solution of aminocyclopyrachlor + triclopyr ester found similar control to the industry standards glyphosate and imazapyr.

DISCUSSION

The hack and squirt technique is an old method and is not perceived to be a contemporary method to kill unwanted trees. In all other applications (e.g. cut stump, basal bark and foliar), large amounts of spray volume or oil dilutions are used to kill trees, often leading to high collateral damage to the surrounding vegetation around the stumps. These applications are also much more costly in terms of herbicides and oil inputs, making them cost prohibitive for large scale applications.. However, with the hack and squirt technique, only 1 ml per 3 inches of trunk diameter showed excellent control of trees 1 YAT. While not 100%, control was enough to make reapplications of herbicides both limited in terms of amount of material needed to achieve 100% control and much more cost effective than cut stump, basal bark and foliar applications.

From our results, we can conclude that aminocyclopyrachlor is an effective tool for use in hack and squirt. However, both of these applications did not achieve 100% control 11 MAT, potentially forcing land managers to retreat any regrowth the following growing season. At both field sites, the stand alone aminocyclopyrachlor treatment controlled trees 91% of the time. However, a 50:50 mixture of aminocyclopyrachlor and

triclopyr amine showed an average of 98% control of trees. These results are surprising considering the apparent physical incompatibility to mix these two herbicides. These two herbicides were not mixed before application because they formed a stringy precipitate when combined and surprisingly this apparently did not occur in the frill cut. The herbicides in the 50:50 mixture were applied separately into the same wound; ½ ml of aminocyclopyrachlor was applied first, followed by ½ ml of triclopyr amine. From the results, it appears that once the herbicides were added to the tree they did not form a precipitate and were absorbed by the tree tissue.

The implications for land and wildlife managers to employ hack and squirt applications are decreased application costs, less collateral damage to their surrounding grasses, forbs and other trees, maintenance of the structural diversity for nesting birds and above all, decreased disturbance of the landscape following cutting and removal of the trees, leading to the inevitable increase in other opportunistic weeds. The down side to the hack and squirt application is that if the desired state after application is for a treeless prairie, then this technique is fundamentally at odds with that desire. Hack and squirt applications would be advantageous when managers are seeking to leave dead trees behind as wildlife habitat or other natural area structure. If a land manager could live with the presence of dead trees on the site and there is no need for their removal, then the hack and squirt technique is a viable option. Another possible avenue for the necessity of hack and squirt applications is the need for immediate control. Field observations at both sites indicated that there was no presence of adventitious shoot development from roots that typically is associated with large scale removal projects of Russian olive trees by the cut stump method. The hack and squirt method may prove advantageous for land managers,

who could leave the dead trees on the site and cut them down at their leisure without the need for costly and extensive retreats of large tracts of cut stump applications.

CONCLUSIONS

From our experiment, we can conclude that aminocyclopyrachlor was an effective herbicide for use in the hack and squirt application methodology. However, no herbicide treatments provided 100% initial control of Russian olive trees 1 YAT.

Aminocyclopyrachlor alone showed 91% control of Russian olive trees within the first year. This level of control was lower than both industry standards glyphosate (94%) and imazapyr (98%). A 50:50 mixture of aminocyclopyrachlor and triclopyr amine provided 98% control of trees 1YAT, even though the two compounds showed incompatibility when mixed. Aminopyralid containing products (e.g. Milestone and Milestone VM+) had lower mean percent control (84% and 89%, respectively), than aminocyclopyrachlor. Triclopyr amine had the lowest percent control when applied alone at both field sites (77% mean control).

LITERATURE CITED

- Bukun, B., R. B. Lindenmayer, S. J. Nissen, P. Westra, D. L. Shaner and G. Brunk. 2010. Absorption and translocation of aminocyclopyrachlor and aminocyclopyrachlor methyl-ester in Canada thistle (*Cirsium arvense*). *Weed Science*. 58: 96-102.
- Clubine, S. 2008. Hack and squirt Verses Basal Bark for Controlling Woody Encroachment of Grassland Quail Habitat. Proceedings of the Joint Meeting for the Society for Range Management and American Forage and Grassland Council, Louisville, KY.
- Deiter, L. *Elaeagnus angustifolia* L. in Bossard, C.C., J.M. Randall, and M.C. Hoshovsky. 2000. Invasive plants of Californias Wildlands. University of California Press, Berkeley CA. pg: 175-178.
- E.I. DuPont de Nemours and Company. 2009. DuPont DPX-MAT 28 Herbicide Technical Bulletin
- Moorhead, D. 2003. Wide-space injection with Arsenal AC herbicide for control of undesirable hardwood stems. Available at: <http://www.bugwood.org/weeds/arsenal.html>
- Parker D., and M. Williamson. 2003. Low-Impact, Selective Herbicide Application for Control of Exotic Trees in Riparian Areas: Salt Cedar, and Siberian Elm. United States Department of Agriculture: Forests Service-Southwest region.
- Sensenman, S.A. 2007. Herbicide Handbook 9th edition. Lawrence, KS: Weed Science Society of America
- Stannard, M., D. Ogle, L. Holzworth, J Scianna, and E Suleaf. HISTORY, BIOLOGY, ECOLOGY, SUPPRESSION OF (*ELAEAGNUS ANGUSTIFOLIA* L.). USDA, NRCS. Boise, ID: USDA, 2002. 1-14.
- Tu, M. 2003. Element Stewardship Abstract for *Elaeagnus angustifolia* L. or , Oleaster. The Nature Conservancy. Available at: <http://www.imapinvasives.org/GIST/ESA/esapages/documnts/elaegang.pdf>

Table 4.1: ANOVA for treatments at both field sites

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	27	1.21108988	0.04485518	5.05	<.0001
Error	82	0.72810602	0.00887934		
Corrected Total	109	1.93919590			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	8	0.01517979	0.01054375	1.32	0.2487
Site	1	0.07632770	0.07632770	8.67	0.0041
Trt	6	0.81131359	0.13521893	11.16	<.0001
Trt*Site	6	0.15867617	0.02644603	2.98	0.0111

Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
MAT 28 v 50:50 MAT 28+ triclopyr ester	1	0.00113604	0.00113604	0.23	0.6347
MAT 28 v triclopyr	1	0.30113985	0.30113985	60.52	<.0001
MAT 28 v aminopyralid	1	0.02273416	0.02273416	4.57	0.0369
MAT 28 v VM+	1	0.02892582	0.02892582	5.81	0.0192
MAT 28 v glyphosate	1	0.00003270	0.00003270	0.01	0.9357
MAT 28 v imazypyr	1	0.00191455	0.00191455	0.38	0.5376
50:50 MAT 28+ triclopyr ester v triclopyr ester	1	0.33926817	0.33926817	68.18	<.0001
50:50 MAT 28+ triclopyr ester v aminopyralid	1	0.03403424	0.03403424	6.84	0.0114
50:50 MAT 28+ triclopyr ester v Milestone VM+	1	0.04152675	0.04152675	8.34	0.0055
50:50 MAT 28+ triclopyr ester v glyphosate	1	0.00155424	0.00155424	0.31	0.5785
50:50 MAT 28+ triclopyr ester v imazypyr	1	0.00010101	0.00010101	0.02	0.8872

Table 4.2: Visual %-Control of Russian Olive for Hack and Squirt Applications

Treatment	Evaluations			
	<u>2011</u>			
	Visual % control (necrosis) ¹			
	Nunn		Wellington	
Aminocyclopyrachlor	84	b-d	98	ab
Triclopyr amine	79	cde	74	e
Aminopyralid	77	de	91	abc
Aminopyralid + triclopyr (Milestone VM+)	88	a-e	90	a-d
50:50 Aminocyclopyrachlor + triclopyr amine	98	ab	99	a
Glyphosate	91	ab	97	ab
Imazapyr	96	ab	99	a
Untreated	0	f	0	f

¹ Means separated by a Fishers protected LSD ($\alpha=0.05$). Means followed by the same letter were not different at the $\alpha=0.05$ level.

Table 4.3: One Factor Contrasts for Aminocyclopyrachlor %-Control of Russian Olive in Hack and Squirt applications

Treatment	Evaluations
	Aminocyclopyrachlor P value ($\alpha=0.05$)
50:50 Aminocyclopyrachlor + triclopyr amine	0.6347
Aminopyrclid	0.0369
10:1 Aminopyrclid + triclopyr amine	0.0192
Triclopyr amine	<0.0001
Glyphosate	0.9357
Imazapyr	0.5376

Table 4.4: One Factor Contrasts for 50:50 mixture of Aminocyclopyrachlor and Triclopyr amine %-Control of Russian Olive in Hack and Squirt applications

Treatment	Evaluations
	50:50 Aminocyclopyrachlor + triclopyr amine
	P value ($\alpha=0.05$)
Aminopyrclid	0.0114
10:1 Aminopyrclid + triclopyr amine	0.0055
Triclopyr amine	<0.0001
Glyphosate	0.5785
Imazapyr	0.8872

Chapter 5: Are European Starlings (*Sturnus vulgaris*) dispersal agents for Russian olive (*Elaeagnus angustifolia L.*)?

ABSTRACT

Two studies were performed to determine if European Starlings disperse Russian olive seeds. In the first study, Russian olive trees were monitored for 1 year at two field sites to determine feeding behaviors of wild animals on Russian olive seeds using two trail cameras (WSCA01 Wing-Scapes Birdcam and a Moultrie MFH-DGS-I60 Game Spy digital camera). In the second study, 20 European Starlings were collected in the field and housed at the USDA-NWRC research facility in Ft. Collins, CO. Birds were fed 25 Russian olive seeds per day and monitored for behavior in individual cages. Seeds that were fed upon were tested for germination and viability and compared to control seeds, hulled seeds, seeds ground on sandpaper and nicked, and seeds soaked in 17.8 M sulfuric acid (H₂SO₄) for 1 hour. From the first study we determined that European Starlings do feed on Russian olive seeds, particularly in November and December. From the second study we determined that Russian olive seeds are actively fed upon by European Starlings in cage trials, with the majority of seeds being regurgitated after 30 minutes.

Digested/regurgitated seeds had the highest level of germination (57%) compared to hulled seeds (40%) and ground/nicked seeds (30%). Viability tests confirmed that 87% digested seeds remained viable after consumption compared to control seeds (76%), hulled seeds (31%), ground/nicked seeds (0%) and acid scarified seeds (0%).

INTRODUCTION

The key aspect of dispersal is the potential for vertebrate animals (e.g. avian and mammalian sources) to distribute seeds from a host plant to distant locations. A key factor for species becoming invasive is the propensity to incorporate other native species into mutualist interactions (e.g. pollination, dispersal) (Bartuszevige and Gorchov, 2006). Benefits to the host plant can include dispersal of seeds away from the parent plant, reducing competition, and inversely causing changes in the germination due to passage of the seed through the digestive tract of the vertebrate (Robertson et al., 2006). Frugivory provides a strong vector by which seeds can be transported to new habitats, increasing the potential invasiveness of the species (Goddard et al., 2009). Passage through the digestive tract can affect seeds in three ways; 1) scarification of the seed coat, 2) removal of germination inhibitors found in the outer pulp, and 3) enhancement of the seeds germination through fecal matter surrounding the seed after defecation (Robertson et al. 2006).

Since its introduction to the United States in the 1900s, Russian olive (*Elaeagnus angustifolia* L.) has escaped from being an ornamental species and spread across many western habitats. Within 100 years of its arrival Russian olive has become the fifth most dominant woody riparian species in the western United States (Friedman et al., 2005). Russian olive is currently found throughout most of the country, except 13 states in the southeast (Katz and Shafroth 2003). Much of the debate over the invasiveness of Russian olive stems from the western states where trees have been observed to be invasive since 1924 (Christensen 1963, Brock 1998, Stannard et al. 2002).

Russian olive trees produce hard-coated seeds that are surrounded by fleshy perianth (Jinks and Ciccarese 1997, Zouhar 2005). Russian olive seeds require a period of afterripening to accomplish a successful level of germination (Katz and Shafroth 2003). This afterripening period may be accomplished in two ways; 1) a period of chilling, or 2) scarification of the seed coat. Hamilton and Carpenter (1975) found that Russian olive seed dormancy was related to a coumarin-like inhibiting substance found in all parts of the seed. Removal of the seed from the exocarp resulted in an increase of 50-60% germination (Jinks and Ciccarese 1997 and Hamilton and Carpenter 1975). Naturally, seeds require an afterripening period of 2 to 3 months around 41° F (5 C) to break dormancy (Zouhar 2005).

The potential for acid scarification to increase the germination of Russian olive has received little attention in the literature. Heit (1967) recommended that Russian olive seeds should be soaked in a concentrated bath of sulfuric acid. Shafroth et al. (1995) acid scarified Russian olive seeds in a concentrated sulfuric acid solution for one hour and found that there was a wide range of variability among their treatments with little net mortality. While these studies have shown that scarification of the outer seed coat is a viable means of inducing Russian olive germination, the natural dispersal mechanism for this tree has not been as rigorously studied.

The scientific literature cites many instances of Russian olive spreading through avian vectors primarily driven by its large production of highly nutritious, viable fruits (Kindschy 1998, Stoleson and Finch 2001, Olson and Knopf 1986, Van Dersal 1939, Borell 1951). It is hypothesized that seeds are ingested along with the fruits, pass through the bird digestive tract, and are deposited in new areas where they can quickly

proliferate (Olson and Knopf 1986). Van Dersal (1939) observed several species of birds that feed upon Russian olive fruits; American Robins (*Turdus migratorius*), Ring-Necked Pheasants (*Phasianus colchicus*), Sharp-Tailed Grouse (*Tympanuchus phasianellus*), Cedar Waxwing (*Bombycilla cedrorum*), Hungarian Partridge (*Perdix perdix*), Northern Bobwhite (*Colinus virginianus*), Western Evening Grosbeak (*Coccothraustes vespertinus*), Valley Quail (*Callipepla californica*), and the Bohemian Waxwing (*Bombycilla garrulus*). Borell (1951) described 28 more birds which feed on Russian olive fruit, in addition to those listed by Van Dersal (1939). However, there is no rigorous scientific study to indicate that birds are actually acting as a dispersal vector. Many of the citations report eyewitness accounts of birds simply perching upon Russian olive trees (Olson and Knopf 1984, Olson and Knopf 1986, Stolson and Finch 2001, Borell 1951, Van Dersal 1939).

The only paper linking dispersal of Russian olive seeds to birds was conducted by Kindschey (1998). In his study, Kindschey examined Russian olive seeds that had passed through the digestive tracts of a flock of European Starlings (*Sturnus vulgaris*). Kindschey (1998) collected 115 excreted Russian olive seeds and 143 whole Russian olive seeds in January after observing a large flock of European Starlings feeding on Russian olive trees. Seeds were kept in their individual groups (excreted or whole) and potted in two large pots filled with a silty-loam soil. Pots were then placed on a windowsill in direct sunlight. Data were collected on date of germination and later the proportion of sprouting seeds was determined. The data showed that there were no statistical difference in the percent germination between seeds that had been digested (10.4%) and seeds not been digested (9.8%) (Kindschey 1998). Many questions were raised

concerning the methodologies and the results of this study and a further examination of Kindschy's findings is warranted.

European Starlings are invasive birds that were introduced into North America in 1890-1891. The Acclimatization Society, a group dedicated to introducing animals featured in William Shakespeare's plays, released 100 Starlings in New York City's Central Park (Cabe 1993). Cabe (2003) concluded that these initial 100 birds were the source of the current population of European Starlings found in North America, currently consisting of over 200 million birds and steadily increasing every year. European Starlings are a stocky, rugged, compact passerine bird with an easily identifiable glossy/shiny coat of dark iridescent feathers, a short/squared tail, a long thin beak, and long pointed wing tips (Cabe 2003). Starling's native ranges stretch throughout much of Eurasia, from Scandinavia in the north to Italy in the south with an eastern edge somewhere east of Lake Baikal in Russia (Cabe 2003). Coincidentally, this range overlaps with the natural range of Russian olive; however there is no information in the literature about Starlings feeding on Russian olive in this range. In North America, European Starlings are typically found in greater abundance in the eastern half of the continent. This is due to better feeding/foraging sites and more acceptable locations for nesting (Cabe 2003). The western United States has a large annual population of Starlings, but their distribution is often interrupted by the presence of mountain ranges (Cabe 2003). Starlings are a migratory bird and vary their geographic locations during two seasonal migrations (September to December and again from mid February to March).

European Starlings have a wide breadth of foods that they feed upon (Cabe 2003, Martinez del Rio et al. 1995, Fischl and Caccamise et al. 1987, Russell 1971). During spring and summer months Starlings are characterized as omnivores, splitting their feeding preference between insects and seeds. Russell (1971) compared the gut contents of 211 birds from across the country and found that 73% of the bird's annual diet consisted of animal material, primarily insects, and in particular 48% of that total was from the order Orthoptera, 36% were Coleopteran, 14% Lepidopteron, and the remainder was a mixture of four other orders. Fischel and Caccamise (1987) observed that of the gut contents of 149 birds were composed of 44.5% plant material, primarily tree (38.1%) and shrub (6.4%) fruits. Contents were also screened for invertebrate components, which were composed of Coleoptera (9.1%), Formiociidae (1.2%), Orthoptera (0.2%), Hemiptera (0.1%), Homoptera (<0.1%), and Molluska (0.7%) (Fischel and Caccamise 1987). During winter months Starlings supplement their diets by changing their gut morphology to feed on berries, grains, seeds, garbage, livestock feed and many other opportunistic feed choices that may present themselves (Cabe 2003). Birds often forage in mixed species flocks with other passerines, including Red-winged Blackbirds (*Agelaius phoeniceus*), Brown-headed Cowbirds (*Molothrus ater*), Common Grackles (*Quiscalus quiscula*), and American Robins (Cabe 2003). Individual birds often require between 7 to 23g of animal based foods (roughly 50 to 60 kcal/day) or anywhere between 80 to 100 g of plant material (roughly 80 to 100 kcal/day) to maintain their metabolism and meet their daily dietary needs (Cabe 2003).

The purpose of this experiment is four fold; 1) to examine the potential for dispersal of Russian olive seeds by European Starlings, 2) Examine the natural feeding behaviors

of these birds by use of advanced wildlife photography cameras, 3) Capture European Starlings in the field and deliberately feed Russian olive seeds to the birds to scarify the outer seeds coat, and 4) Compare biologically scarified Russian olive seeds to several test groups; Control seeds not removed from their pulp, seeds that have been removed from the pulp, seeds that have been scarified with a solution of sulfuric acid, and seeds that have been mechanically ground but not scarified to artificially simulate the gizzard of a bird.

MATERIALS AND METHODS

Seed Collections

Russian olive seeds were collected from four sites across northern Colorado; Nunn, Greeley, Wellington Number 4 reservoirs in Wellington, and Ft. Collins. In August to September 2010, 1000 seeds were hand collected. Seeds were collected at random from 50 different trees on each site. Seeds were stored in four separate plastic totes and placed in a 4.4 C (40 F) cold room. Seeds were only removed from the cold room during periods of testing, transportation, or germination.

Game camera study

This study was carried out at two field sites (Nunn, CO and the Wellington Number 4 reservoir near Wellington, CO). At both sites, 9 ft U-posts were pounded into the ground, at least 1 ft into the soil, facing a single Russian olive tree at an optimal performance distance of 4 ft measured from the trunk of the tree. Two trail cameras were fixed to the U-post by means of wooden brackets that could be adjusted along the height

of the U-post by metal plates fastened to U-bolts. Cameras were positioned at two elevations (1 ft from the top of the U-post and 1 ft from the soil surface).

The top camera was a motion activated WSCA01 Wing-Scapes Birdcam. The camera was designed to capture the feeding habits of any birds that flew into the upper portions of the Russian olive trees. The camera was set to capture 8 mega pixel high resolution still life images set for three image bursts when movement was detected. Pictures were captured only during the day as camera did not operate at night. The lower camera was a Moultrie MFH-DGS-I60 game spy digital camera set 1 ft above the soil to capture small mammals and birds that foraged at ground level. This camera was set to operate in both the day and night to capture 6 mega pixel high resolution pictures of any mammals or birds that were detected in a 25 ft arch of the camera. Both cameras were powered by batteries and supplemented with solar panels. All images were stored on removable 4GB SD media cards.

Photos were analyzed for any bird or mammals species. Data were collected on species identification and any other visual observations pertaining to feeding that could be discerned from the pictures. The experiment was carried out over a 1 year period, with cameras checked weekly for photos and maintenance. Cameras were moved after a 4 week period to a new location on the site, following the same methods.

Bird feeding study

Both federal (permit MB019065) and state (permit: 10TRb2006) permits were acquired prior to testing. On December 14th, 2010 20 European Starlings were captured at a cattle feedlot outside of Platteville, CO (40°12' 39.84"N by 104°52' 15.84"W) using

modified Australian Crow traps. Traps were constructed of a steel cage, surrounded by chicken wire that was 10 ft wide by 30 ft long by 6 ft tall. The trap was subdivided near the far end to create two separate areas for the birds to congregate. Traps caught European Starlings and Red-Winged Blackbirds inside of the chicken wire coop by means of a one way entrance that prevented the birds from escaping once they had entered. Birds were captured at the feedlot due to their high abundance in the fall months congregating near the lot to feed. Trained ornithologists from the NWRC supervised the capture of the Starlings. Entrance to the cages was permitted by two doors, found on the far ends of the cage that allowed access for up to two people at a given time. Once inside of the cage, birds were captured using hand nets to pin them against the walls of the cage and then transferred to group transport boxes (1 × 0.6 × 0.3 m), up to 10 birds per box.

Starlings were transported by vehicle, in well-ventilated transport boxes, up to 10 birds per box. Transport boxes were protected from extreme temperatures (e.g., <0 °C, >30 °C), direct sunlight, and precipitation to prevent stress on the animals as they were being transported. All birds were transported to the NWRC Outdoor Aviary Research Facility (OARF) and housed in individual testing cages (0.9 m × 1.8 m × 0.9 m) in Building 25. Cages were open on the top, through a wire mesh, and were closed with sheet metal on the bottom. Three doors were positioned along one end of the cages. Birds entered through the middle door, which was hinged and closed with a push-pin latch. A maintenance diet was provided daily (*ad libitum*) to all birds. For Starlings the maintenance diet consisted of pelletized feed. Water was also provided daily (*ad libitum*) to all birds throughout the study. Both food and water were simultaneously presented to

the birds through the left hand door of the cage, which was a vertical sliding piece of sheet metal.

Russian olive fruits were presented to the birds in free choice. In free choice feeding 25 fruits at a time were presented in a small clay dishes, through the right hand door of the cage and left for 24 hours. The birds were given the option to freely feed on the seeds at their leisure. Depending upon the bird feeding behaviors, each bird was held until it had consumed a total of 50 seeds for the test, or a grand total of 1000 seeds. Free choice testing was carried out over a 1-week period. Water, maintenance diet, and test fruits were checked every morning at 0700 hours (7:00 am MST).

Feeding was monitored using a camcorder mounted on a tripod facing the cages for further analysis on feeding behaviors. Video cameras were positioned either on tripods on top of the cage or were attached to the sides of the cage. Video was recorded over an 11 hour time period, just after seeds were introduced to the birds in the morning. Video was screened after the testing to determine feeding behaviors.

Paper tray liners were removed from the cages every other day for seed collections from the fecal matter and regurgitations. Seed passage was determined by screening the feces and regurgitated seeds for those that had been stripped of the fleshy perianth, which constituted a “cleaned” seed. Seed passage was determined by screening the feces and regurgitations by collecting the respective sample from fecal trays. Russian olive seeds were identified from the feces and distinguished from other seeds consumed. Seeds were considered scarified if they were defecated or regurgitated, based upon their morphology and the digestive processes that occurred.

On completion of the study all Starlings were euthanized by CO₂ asphyxiation, in accordance with American Veterinary Medicine Association (AVMA) standards.

Euthanized birds and birds that died during the study were incinerated.

Seed Testing

Seed feed values were tested by Ward Labs⁷ for multiple feed and nutrient factors; % crude protein, % crude fiber, % ash, N-free extract (% carbs), ether extract (% fat), % starch, % calcium, % phosphorus, % potassium, manganese, iron, and relative energy value (Mcal/cwt). Values were compared to known feed values of sunflower (*Helianthus annuus*), proso millet (*Panicum miliaceum*), grain barley (*Hordeum vulgare*) and grain sorghum (*Sorghum bicolor*) found in the National Academy of Sciences Atlas of Nutritional Data on United States and Canadian Feeds (1971) (Table 1).

Starling digested seeds were tested for germination and compared to six other test seeds. Control seeds received no treatment and maintained the outer fleshy seed pulp. Seeds removed from the pulp (hulled) were soaked in 250ml of distilled (DI) water for 24 hours then removed of their saturated fleshy pulps. Scarified seeds were soaked in a concentrated 17.8 M sulfuric acid (H₂SO₄) solution for 1 hr before being removed from the acid and washed for 5 minutes under DI water. Seeds that were ground were soaked in DI water for 24 hours then removed from their outer seeds coats. Seeds were allowed to air dry for 20 minutes. Seeds were then placed into a petri-dish, whose surface was covered with 80 grit sand paper. The lid of the petri-dish was overturned and also covered in 80-grit sand paper. Seeds were then hand ground for a 5 minute interval using a back and forth motion of the two petri-dish covers. Once seeds were ground, they were

⁷ Ward Laboratories, 4007 Cherry Ave., P.O. Box 788, Kearney, NE 68848, Tel: 1800-887-7645

nicked along one edge of the pointed ends of the seed coat using a box cutter. All seeds were surface sterilized with a 10% household bleach (sodium hypochlorite or NaClO) solution over night. Seeds were positioned on soaked germination paper sheets, 40 seeds to a sheet. Seeds were then covered with another soaked germination paper, rolled up and placed into plastic sealable bags. Seeds were placed into a 22.5 C growth chamber. Seeds were checked weekly for emergence of the hypocotyls, indicating germination. Once deemed germinated, seeds were removed from the germination paper to prevent double counts (Table 5.4). Data for % germination were checked for normality and transformed by square root then subjected to an analysis of variance using SAS version 9.2 using a PROC GLM procedure (Table 5.1). Transformed means were separated by Fishers protected LSD ($\alpha=0.05$) but are presented in the original scale.

After a 6 week germination period, seed viability was determined using a Tetrazolium (TZ) test (Colorado Seed Lab⁸). Lots of 60 seeds were replicated three times for all seven test groups. Seeds were removed of their outer pulp and seed coats were nicked. Seeds were then imbibed in water for 24 hours. After soaking, a small slice of the seed embryo was removed and put in a TZ solution for 24 hours. During the soaking the live embryos turned red. Embryos were examined under a microscope and viability was determined visually as viable or not. Data for % viability were checked for normality and transformed by square root then subjected to an analysis of variance using SAS version 9.2 using a PROC GLM procedure (Table 5.2). Transformed means were separated by Fishers protected LSD ($\alpha=0.05$) but are presented in the original scale.

⁸ Colorado Seed Laboratory, Dept. of Soil & Crop Science, Colorado State University, Ft. Collins, CO 80523

RESULTS

Game camera study

For the Wing-Scapes birdcam, European Starlings were observed feeding upon Russian olive seeds in late November/early December (Figure 5.1 and 5.2). Birds exhibited a flocking feeding behavior and fed upon seeds en mass. At maximum, one picture observed 18 different Starlings feeding upon a single tree at 1604 hours (4:04 pm MST). From other pictures it appeared that Starlings typically fed upon seeds from 1500 hours to 1645 hours (3:00pm to 4:30pm) with one occurrence of feeding in the morning at 0940hours (9:40 am). Other birds observed in the Russian olive trees, but not observed feeding upon the seeds included the American Robins (*Turdus migratorious*), Northern Flicker (*Colaptes auratus*), American Crow (*Corvus brachyrhynchos*) and a House Finch (*Carpodacus mexicanus*).

For the Moultrie camera, European Starlings were observed feeding on the ground for presumably Russian olive seeds. Several pictures showed birds scratching and digging into the snow searching for seeds. A high proportion of pictures taken were, however, of Cottontail Rabbits (*Sylvilagus* Spp.). Other animals seen searching under Russian olive trees included the Coyote (*Canis latrans*), Mule deer (*Odocoileus hemionus*), Magpie (*Pica hudsonia*), and Skunk (*Mephitis mephitis*).

Bird feeding study

During testing three birds expired before Russian olive seeds were presented to them. For the remaining 17 birds, video recordings were made to monitor Starling feeding behaviors on Russian olive seeds. Analysis showed that Starlings, on average,

consumed seven seeds within the first few minutes of seeds being introduced into their food bowls. Birds would be immediately attracted to the Russian olive seeds and quickly consumed them. Within 2 days of testing, European Starlings had consumed 850 seeds. Analysis of the video confirmed that the majority of seeds fed upon by Starlings are regurgitated approximately 30 minutes after consumption. The video analysis concluded that birds exhausted their daily Russian olive seed allotment (25 seeds/bird/day) within 4 hours.

Seed testing

Germination of Russian olive seeds indigested by European Starlings was higher than all of the other test groups (57%) followed by the hulled seeds (40%) and the ground/nicked seeds (30%). None (0%) of the control seeds or seeds subjected to the sulfuric acid treatment (1 hr) germinated.

DISCUSSION

Kindshey (1998) performed a study in which he compared the percent germination of digested (10.4%) and undigested (9.8%) Russian olive seeds to one another and found no statistical difference. From our results, we have shown that European Starlings may disperse Russian olive seeds and can stimulate the germination (57%) of seeds that have been fed upon compared to undigested seeds (0%). We have also shown that European Starlings may act as a potential dispersal vector, in that dispersal is inherent following consumption. However, our study does not physically link

dispersal of Russian olive seeds to European Starlings, but does show the inherent ability of the birds to possibly disperse Russian olive seeds.

Comparisons between Russian olive and European Starling winter distribution maps of the United States (Figures 5.1, 5.2, 5.3, and 5.4) show that both species occupy the same geographic regions. European Starlings congregate in the eastern United States during the breeding season, but shift their population to span the entire country during the winter months (Figure 5.3 and 5.4). This shift during the winter months overlaps with the much higher abundance of Russian olive trees in the 17 western states where Russian olive is of greater abundance due to favorable climatic variables. This overlap in occurrence may further support the potential for European Starlings to act as a dispersal vector for Russian olive during the winter months. Due to the high carbohydrate content of Russian olive fruit, European Starlings may be attracted to these nutrient rich fruits as they are traveling into the western states thus acting as a dispersal agent. Further testing is warranted to assess the true implications of dispersal and to document the physical transport of seeds away from the parent plant, but our research has indicated both the possibility and the observance of European Starlings feeding upon Russian olive seeds. We observed European Starlings regurgitating Russian olive seeds after 30 minutes following consumption in the controlled study. Using this average time, we can calculate the maximum distance that a European Starling may disperse a Russian olive seed following consumption. European Starlings have an average flight speed of 60 to 80 km/hr (37 to 50 MPH) (Cabe 2003), indicating that they may have a maximum dispersal distance of 30 to 40 km/½ hour or 19 to 25 radial miles from the origin.

Several key observations were made from our experiments on the behavior of European Starling feeding. From our game camera study to collect feeding behavior of European Starlings, over 30,000 pictures were captured for both sites. A high proportion of these pictures were a result of wind triggering the shutter of the lenses by moving grasses or limbs. However, it became obvious in the late fall/early winter that European Starlings seemed to congregate in the branches of Russian olive trees, actively feeding upon the seeds still on the branches and the seeds that had fallen to the ground. From the Bird cam pictures, birds seemed to flock on trees with visible fruit and fed continuously over a 4 week period from December 11th through January 7th. Birds appeared to congregate in Russian olive trees to feed on the abundant fruits (Figure 5.1). Observations of the photos also indicated that birds will consume the fruit and appear to disperse it away from the parent plant (Figure 5.2). From January 7th onward, birds were detected by the Moultrie camera more frequently feeding on the soil surface, searching through the snow looking for fruits. Birds appeared to have cued in on the availability of the Russian olive fruits on the soil surface, and appeared to be continually attracted to those fruits even after snow had covered the ground.

From our feeding study, it was originally hypothesized that Starlings would feed upon Russian olive fruits and then defecate the digested seeds. Some seeds exhibited a darkened exterior, indicating that they had been digested and passed through the whole digestive tract of the birds. However, these seeds were rare and accounted for a small portion of the whole. Analysis of the video determined that the majority of seeds fed upon by European Starlings were regurgitated within a 30 minute window of feeding. Birds would consume between seven to nine seeds within the first of couple minutes of

seeds being introduced in the morning. After 30 minutes, birds would be observed regurgitating Russian olive seeds, followed by consumption of one or two more seeds. On average, birds had consumed the 25 seeds allotted to them within 4 hours. When collected, seeds were observed to be completely removed of the outer fleshy pulp, leaving the stripped hard seed. This lack of fleshy pulp indicates that the birds are digesting off the easy to remove pulp and then expelling the hard seed. This digesting is possibly occurring in the bird's proventriculus, or glandular stomach, where acid and digestive enzymes breakdown food before it is passed to the gizzard, or muscular stomach, where physical abrasion of food occurs. Viability testing from TZ analysis indicated that seeds that had been digested by European Starlings were still viable (87%) and potential scarification inside of the bird had not damaged the embryo (Table 5.5). The digestive tract of the bird may exhibit only a limited affect on the developing seed and may actually only dissolve off the germination inhibitor, thus acting not only as a dispersal mechanism but as a possible germination initiator. Control seeds (76%) and hulled seeds (31%) were the only other viable seeds after TZ testing, indicating that any invasive treatments to the embryo (e.g. ground/nicked, and the sulfuric acid treatments all with 0% viability), may damage the developing embryo and limit dispersal.

Another possible explanation for these results may explain the difference in germination and viability of the ground/nicked seeds. All seed treatments had severe fungal growth during the six week germination trials, even after soaking the seeds in a 10% bleach solution for 24 hours. The germination data for Russian olive seeds was collected weekly and then averaged once the experiment was concluded after six weeks to gain an average germination; however, if we look at a weekly account of germination,

we saw that a high proportion of the ground/nicked seeds germinated early in our timecourse, similar to the regurgitated/digested and the hulled seeds. This trend began to decrease everyweek, until at five weeks there was no germination of the ground/nicked seeds. This possibly was due to fungal growth penetrating into the inner core of the seed and damaging the developing Russian olive embryo.

Scarification of the outer seed coat was not successful using 17.8 M sulfuric acid for 1 hour. Heit (1967) recommended that Russian olive seeds should be soaked in a concentrated bath of sulfuric acid. Shafroth et al. (1994) acid scarified Russian olive seeds in a concentrated sulfuric acid solution for 1 hour and found that there was a wide range of variability among their treatments with little net mortality. However, neither of these studies mentioned the molarity of sulfuric acid that they were using and list it only as “concentrated.” We can conclude from our study that 17.8 molar sulfuric acid may, in fact, be too concentrated and a lower molarity is warranted.

Comparing the germination rates between our test groups, we saw that seeds that had been consumed by European Starlings exhibited a similar germination rates as seeds that had been hulled and seeds that had been ground/nicked. Both the hulled and the ground seeds had been removed of the outer fleshy pulp similar to the digested seeds. Hamilton and Carpenter (1975) found that Russian olive seed dormancy was related to a coumarin-like inhibiting substance found throughout the outer seed covering. Removal of the seed from the exocarp resulted in an increase of 50-60% seed germination (Jinks and Ciccarese 1997 and Hamilton and Carpenter 1975). This germination inhibitor may act as a natural dispersal mechanism, in which seeds must be consumed by animals to digest off the pulp and in turn transported to a new location away from the parent where

they can spread to new areas. However, Russian olive seeds have a much lower net energy level (1659 Kcal /kg) compared to other more apparent nutritious sources (Table 5.3). Feed values for the seed indicated that it has a low % crude protein and % fat level compared with other seeds but a high level of % carbohydrates. This fact may further increase the notion of Russian olive being a usable winter feed source, simply from the fact that it is primarily the only remaining seed crop available in the late winter months.

CONCLUSION

Combining our observations from the Russian olive and Starling distribution maps, trail cam study, feeding study and germination/viability trials all indicate that European Starlings may possibly act as a dispersal agent for Russian olive in that they are both attracted to Russian olive fruits in the wild and feed upon them with vigor. Further study of this mechanism is warranted to fully assess the seed shadow of birds, such as the European Starling, to fully understand the relationship between wildlife and invasive species dispersal.

LITERATURE CITED

- Bartuszevige, A.M., and D.L. Gorchov. 2006. The relative importance of landscape and community features in the invasion of an exotic shrub in a fragmented landscape. *Ecography*. 29(2): 213-222.
- Borell, A. E. 1951. Russian olive as a Wildlife Food. *Journal of Wildlife Management* 15: 109-110
- Brock, J.H. 1998. Invasion, Ecology, and Management of *Elaeagnus angustifolia* (Russian olive) in the Southwestern United States of America. 123-136. In U. Starfinger, K. Edwards, I. Kowarik, and M. Williamson. *Plant Invasions: Ecological Mechanisms and Human Responses*. Backhuys Publishers, Leiden, The Netherlands
- Cabe, P.R. 1993. European Starling (*Sturnus vulgaris*). *The Birds of North America*. 48: 1-23
- Christensen, E.M. 1963. Naturalization of Russian olive (*Elaeagnus angustifolia* L.) in Utah. *American Midland Naturalist*. 70-1: 133-137.
- Fischl, J., and D.F. Caccamise. 1987. Relationships of Diet and Roosting Behavior in the European Starling. *American Midland Naturalist*. 117 (2): 395-404.
- Friedman, J. M., G. T. Auble, P. B. Shafroth, M. L. Scott, M. F. Merigliano, M. D. Freehling, and E. R. Griffin. 2005. Dominance of Non-Native Riparian Trees in Western USA. *Biological Invasions* 7: 747-751.
- Goddard, R.H., Webster, T.M., Carter, R., and Grey, T.L. 2009. Resistance of Benghal dayflower (*Commelina benghalensis*) seeds to harsh environments and the implications for dispersal by Mourning doves (*Zenaida macroura*) in Georgia, U.S.A. *Weed Science* 57: 603-612.
- Hamilton D.F., and Carpenter, P.L. 1975. Regulation of seed dormancy in *Elaeagnus angustifolia* by Endogenous Growth Substance. *Canadian Journal of Botany* 54: 1068-1073.
- Heit, C.E. 1967. Propagation from seed part 6: Hardseededness-a critical factor. *American nurseryman* 125: 10-12, 88-96.
- Jinks, R.L., and Ciccarese, L. 1997. Effects of Soaking, Washing and Warm Pretreatment on the Germination of Russian olive and Autumn Olive Seeds. *Tree Planters Notes*. Winter/spring 1997: 18-23.
- Katz, G L., and P. B. Shafroth. 2003. Biology, Ecology and Management of *Elaeagnus angustifolia* L. *Wetlands*. 23: 763-777.

- Kindschy, Robert R. 1998. European Starlings Disseminate Viable Russian-Olive Seeds. *Northwestern Naturalist*. 79: 119-20.
- Martinez del Rio, C., K.E. Brugger, J.L. Rios, M.E. Vergara, and M. Witmer. 1995. An Experimental and Comparative Study of Dietary Modulation of Intestinal Enzymes in European Starlings (*Sturnus vulgaris*). *Physiological Zoology*. 68(3): 490-511.
- Olson, T. E., and F. L. Knopf. 1984. Naturalization of Russian-olive implications to rocky mountain wildlife. *Wildlife Society Bulletin*. 12: 289-298.
- Olson, T. E., and F. L. Knopf. 1986. Naturalization of Russian-Olive in the Western United States. *Western journal of applied forestry* 1: 65-69.
- Robertson, A.W., Trass, A., Ladley J.J and Kelly, D. 2006. Assessing the benefits of frugivory for seed germination: the importance of the deinhibition effect. *Functional Ecology*. 20: 58-66.
- Russell, D.N. 1971. Food Habits of the Starling in Eastern Texas. *The Condor*. 73(3): 369-372
- Shafroth, P.B, G.T. Auble, and M.L.Scott. 1995. Germination and establishment of native plains cottonwood (*Populous deltoides marshall subsp. monilifera*) and exotic russian-olive (*Elaeagnus angustifolia* L.). *Conservation Biology*. 9: 1169-1175
- Stannard, M., D. Ogle, L. Holzworth, J Scianna, and E Suleaf. HISTORY, BIOLOGY, ECOLOGY, SUPPRESSION OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA* L.)L.)L.). USDA, NRCS. Boise, ID: USDA, 2002. 1-14.
- Stoleson, Scott H., and Deborah M. Finch. 2001. Breeding Bird Use of and Nesting Success in Exotic Russian olive in New Mexico. *The Wilson Bulletin*. 113: 452-55.
- Van Dersal, and William R. 1939. Birds that feed on Russian olive. *The Auk*. 56: 483-84.
- Zouhar, K. 2005. *Elaeagnus angustifolia*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/>

Table 5.1: ANOVA for germination tests

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	6	152.2713080	25.3785513	5.53	0.0153
Error	8	36.7088154	4.5886019		
Corrected Total	14	188.9801233			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	2	15.4647555	7.7323778	1.69	0.2451
Trt	4	136.8065525	34.2016381	7.45	0.0083

Table 5.2: ANOVA for TZ viability tests

Source	DF	Squares	Mean Square	F Value	Pr>F
Model	6	245.4336512	40.9056085	72.93	<.0001
Error	8	4.4877864	0.5609733		
Corrected Total	14	249.9214377			

Source	DF	Type III SS	Mean Square	F Value	Pr>F
Rep	2	1.6944057	0.8472028	1.51	0.2777
Trt	4	243.7392456	60.9348114	108.62	<.0001

Table 5.3: Relative feed values for Russian olive

	Relative Feed Values				
	Russian Olive	Sunflower*	Proso Millet*	Grain Barley*	Grain Sorghum*
% Crude Protein	4.6	17.9	12.8	13.0	10.0
% Crude Fiber	5.7	31.0	7.2	6.0	2.6
% Ash	4.7	3.3	3.0	3.4	2.4
N-free extract (% Carbs)	83.8	20.1	73.0	75.7	81.5
Ether Extract (% fat)	1.2	27.7	4.0	1.9	3.5
Starch %	26.5	Not Available	Not Available	72.0	Not Available
Calcium %	0.22	0.18	0.04	0.08	0.03
Phosphorus %	0.07	0.56	0.34	0.45	0.32
Potassium %	1.52	0.71	0.48	0.55	Not available
Manganese	9 ppm	23.10 ppm	Not available	8.9 ppm	Not available
Iron	145 ppm	300 ppm	800 ppm	900 ppm	Not available
Energy	1659 Kcal /kg	Not available	3316.0 K cal/kg	2960 K cal/kg	3810 K cal/kg

⁸ Values collected from National Academy of Sciences Atlas of Nutritional Data on United States and Canadian Feeds (1971)

Table 5.4: % germination for bird feeding study

Treatment	Evaluation	
	% Germination *	
Digested seed	57	a
Hulled seed	40	a
Whole seed (Control)	0	b
Ground/Nicked seed	30	a
Sulfuric acid ¹ (1hr)	0	b

¹Sulfuric acid (H₂SO₄) molarity:17.8

*Means separated by Fishers protected LSD ($\alpha=0.05$). Means followeb by the same letter were not different at the $\alpha=0.05$ level.

Table 5.5: % viability for bird feeding study

Treatment	Evaluation	
	% Viability (TZ)*	
Digested seed	85	a
Hulled seed	31	b
Whole seed (Control)	76	a
Ground/Nicked seed	0	c
Sulfuric acid ¹ (1hr)	0	c

¹Sulfuric acid (H₂SO₄) molarity:17.8

*Transformed means separated by Fishers protected LSD ($\alpha=0.05$) but are presented in their original scale. Means followed by the same letter were not different at the $\alpha=0.05$ level.

Figure 5.1: NIISS image for Russian olive distribution in the United States

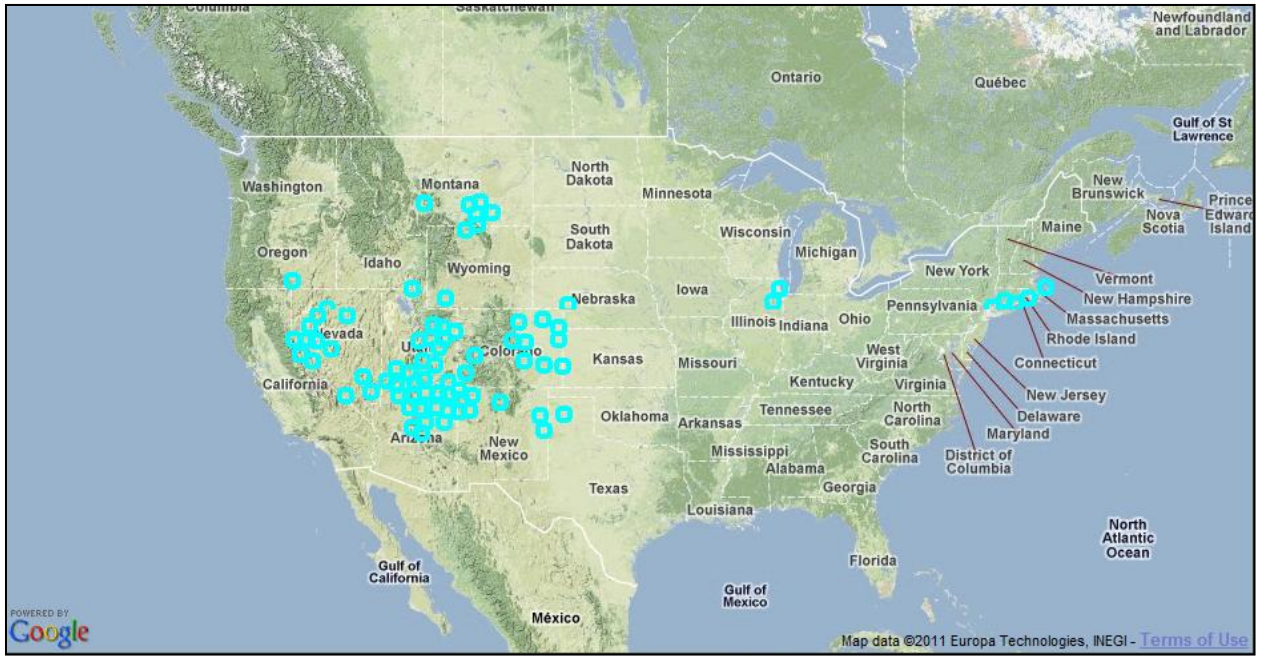
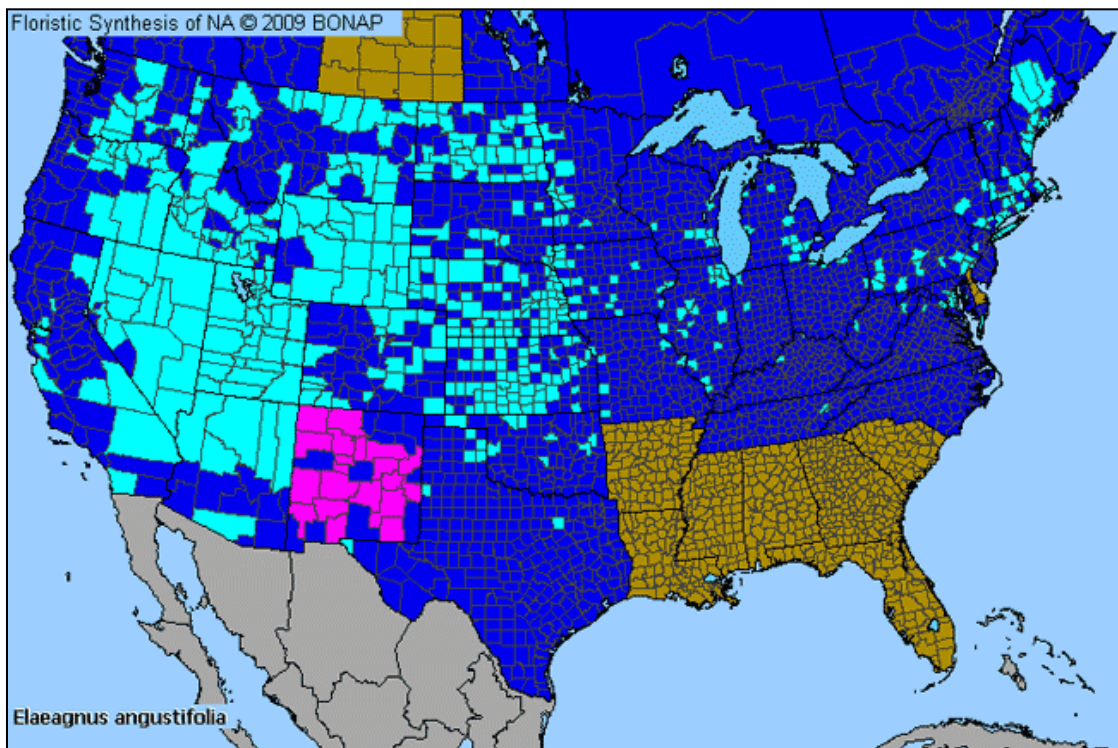


Figure 5.2: Pollen Library.Com presence/absence map for Russian olive in the United States and Canada



Map color description	
■	Unreported (Absent for area)
In State map	
■	Present/Native
■	Adventive or Introduced/Native
■	Present/Exotic
In County map	
■	Present in state/Native (Native and Present in state, but not Present in a county)
■	Present in county/Native (Native and Present in state, and Present in county)
■	Present in state/Exotic (Exotic and Present in state, but not Present in a county)
■	Present in county/Exotic (Exotic and Present in state, and Present in county)
In both State and County maps	
■	Extinct or possibly extinct
■	Extirpated/Historic
■	Rare
■	Noxious
■	Eradicated
■	Waif (Exotic plant found in the wild and generally unable to reproduce on its own; thus never becoming established or part of the naturalized flora)
■	False geographic report
■	Questionable Presence (cross-hatched)
In regions & Zones maps	
	Provides background color for specific regions/zones selected

Figure 5.3: Summer distribution of European Starling across the United States and Canada

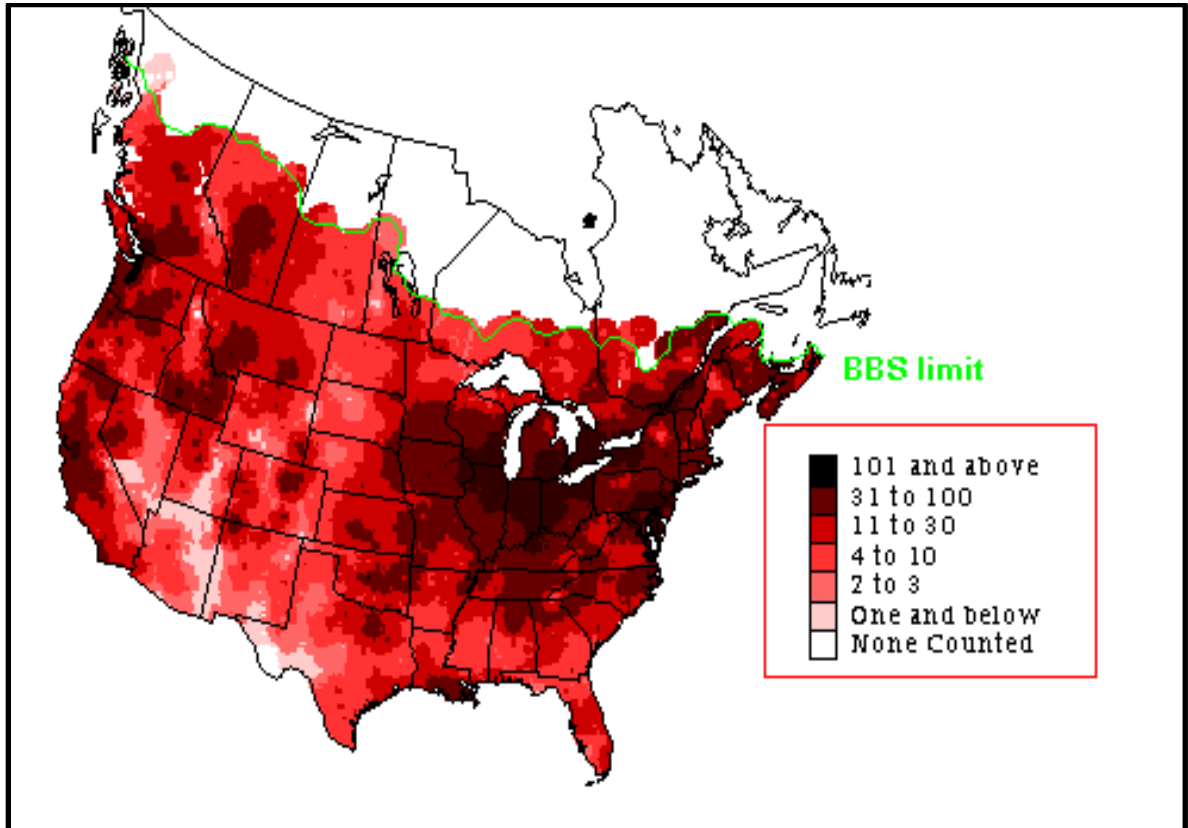


Figure 5.4: Winter distribution of European Starling across the United States and Canada

