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PLANT MATERIALS TECHNICAL NOTE

Russian Olive *Elaeagnus angustifolia* L.
Effect of Seed Burial Depth on Seedling Emergence and Seed Viability

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Russian olive fruit on the soil surface after dispersal from the tree

Introduction

Russian olive *Elaeagnus angustifolia*, is a non-native tree widely-used for windbreaks and shelters, but has vectored across the landscape by animals and water and infested many eastern Montana and Wyoming riparian areas. It is considered a noxious weed in Wyoming and a regulated plant in Montana. With a combination of shade tolerance, high seed viability, annual fruit production after 10 years of age (Lesica and Miles, 2001), and transport of the seeds by animals and water; Russian olive can infest a riparian area rather quickly when compared to native trees.
In riparian areas and flood plains, solid stands of Russian olive have been removed and herbicides applied to the stumps (USDA Forest Service – Southwestern Region, 2012). Little is known, however, about subsequent re-invasion or the factors influencing the successful re-establishment of this woody species. Tree removal creates openings for the regeneration of Russian olive from the various seed vectors previously mentioned, and most importantly, the existing seed bank in the soil. In an Arizona study, (Brock, 2003) found the Russian olive seed bank was composed of 63% new and viable seeds, while the remaining 37% were considered old and with low or no germination potential. Conversely, 28 year old Russian olive seeds kept in a storage facility (averaging 55° to 60° F. year round and approximate relative humidity measuring 20% to 30%) had a viability of 77% (Scianna et al., 2012). Free-flowing river deposition may alter Russian olive seed bank establishment dynamics by burying seeds under sediment and increasing seed demise from soil pathogens and excess moisture.

Objectives

The objectives in this study were to determine: 1) if seed burial depth influenced the emergence of Russian olive seedlings, 2) if non-emerged seed remained viable at the end of the study, and 3) if emergence was related to the population source, i.e., seeds collected from upland populations (windbreaks) versus invasive, naturally-occurring riverine (riparian) populations.

Materials and Methods

Russian olive seeds (see Figure 1) were collected from trees in the fall of 2012 from four planted upland sites and four invaded riverine sites along the Yellowstone River, near Miles City and Sidney, Montana. The Riverine (riparian) collection sites were East Yellowstone, Elk Island, Roche Jaune, and Seven Sisters. The planted sites were Iverson, Love Street, Radar Site, and Westside. Sample lots of 200 seeds from each site were sent to the Montana State Seed Testing Lab in Bozeman, Montana, in 2013 for tetrazolium viability testing. Because of the high viability results (see Chart 1) for all lots, only one seed was planted per each 7-cubic-inch Conetainer™. The planting depths tested were 1, 3, and 5 inches.

The soil used as a propagation medium was a Yellowstone River bank sandy loam, composed of 63% sand, 30% silt, and 7% clay (Soil Survey Staff, 1998). It was collected from a site where Russian olive was removed near the East Yellowstone collection area. The experimental soil was typical of the field soils from riparian zones infested with Russian olive.

Three hundred seeds from each site were placed in 0.5 kilograms of soil, 100 ml of water added, and then stored in a walk-in cooler for a cold-stratification period of 75 days in order to break seed dormancy (USDA-Forest Service, 2008).

Upland and riverine populations were randomized into blocks arranged by burial depth treatment and replicate (see Figure 2). Twenty-five seeds from each of the eight sites were planted per replication for a total of 200 seeds per replication. With four replications per treatment, a total of 800 seeds were planted for each depth treatment, for a total of 2,400 seeds for the entire study. In order to simulate moist riparian soil conditions in the greenhouse, the containers were watered once per day to field capacity. The greenhouse was maintained between 68º to 78º F. during the day, 65º to 70º F. during the night, and on 16-hour photoperiods, but when summer temperatures were high, the containers were watered twice daily. Emergence, when seedling cotyledons broke through the soil surface (see Figure 3), was counted and recorded a minimum of three times per week.

Figure 1. Seed from the Seven Sisters site

← N

Rep 4
Rep 3
Rep 2
Rep 1

5-inch burial

Rep 4
Rep 3
Rep 2
Rep 1

3-inch burial

Rep 4
Rep 3
Rep 2
Rep 1

1-inch burial

Figure 2. Greenhouse setup diagram

After 188 days from planting, all containers were emptied and each non-emerged seed was inspected for viability through ocular observation and testing for firmness by squeezing the seed between two fingers (see Figure 4). If the seed was discolored and soft, it was considered rotten and inviable. If the seed was firm, it was then taken to a microscope and excised with a scalpel to determine if the embryo was white and firm, i.e., viable (Borza et al., 2007).

Emergence data collected from the 1-inch burial treatment were analyzed using Bartlett's test of Equal Variances (Statistix 8.2) with a significance level of $p = 0.05$. Differences in the distribution of live-emerged seeds, live-non-emerged seeds, and dead-non-emerged seeds (see Figure 4) from the 1-inch depth among the two populations were tested using nominal logistic regression (JMP 10.0.2) with a significance level set at $p = 0.05$.

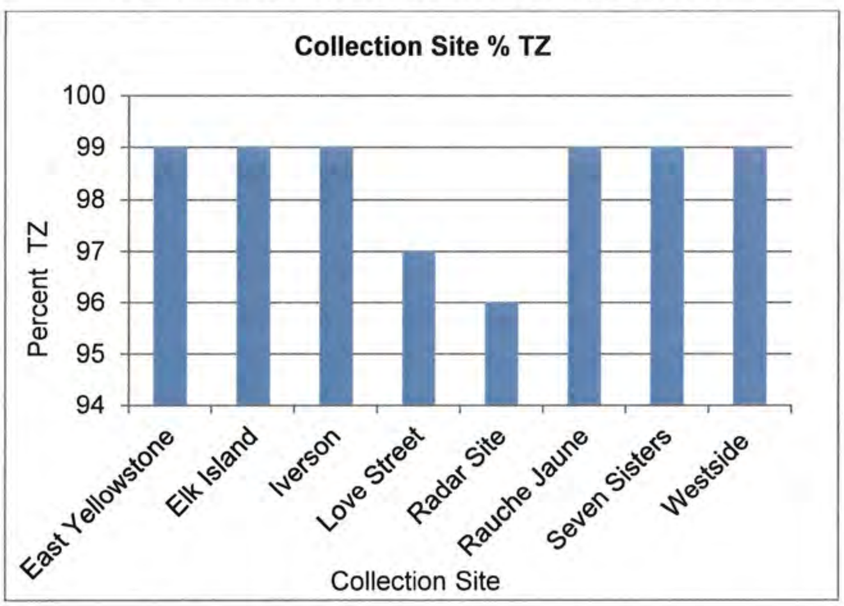
Figure 3. A Russian olive cotyledon emerging

Figure 4. A dead Russian olive seed

Results

The tetrazolium tests of seeds from trees from the eight collection sites ranged from 96% to 99% and had a mean viability of 98.4% (see Chart 1).

Chart 1. Russian olive seed tetrazolium (TZ) results



The first seedling emerged from the 1-inch depth eight days after seeding. Infrequent emergence at that depth was still occurring at the time the trial ended 188 days later. Variation in the hardness of the Russian olive seed coat may have contributed to the time lapse in germination (Young and

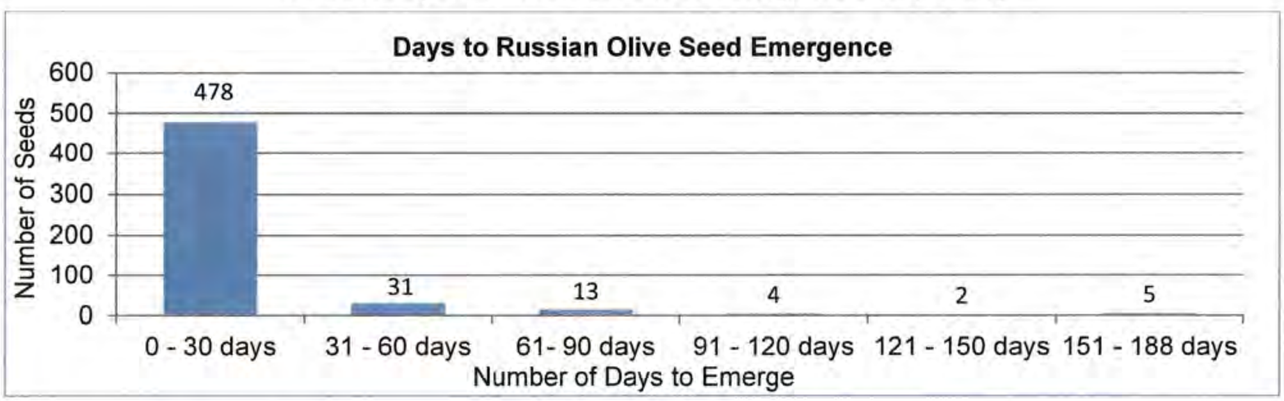
Young, 1992). The study concluded with a total of 533 seeds emerged from all depths (see Table 1). Of that total, 532 of 800 seeds emerged from the 1-inch depth (66.5%) and 1 of 800 seeds emerged from the 3-inch depth (0.00125%). Of the 533 seeds that emerged, 478 (89.6%) emerged within 30 days of planting (see Chart 2). The remaining emergence occurred in the following intervals: 31 to 60 days - 31 (5.8%); 61 to 90 days - 13 (2.5%); 91 to 120 days - 4 (0.75%); 121 to 150 days - 2 (0.375%); and 151 to 188 days - 5 (0.9%). The lone emergence at the 3-inch depth may have been the result of planting less than 3 inches deep. No seedlings emerged from the 5-inch depth. Live roots, however, were observed several weeks after planting in the slits at the bottom of several containers in the 5-inch treatment, indicating some seeds had germinated. These roots, however, were not visible (alive) when the study ended.

Table 1. Russian olive seed germination and emergence from 1-, 3-, and 5-inch burial depths

Collection Site	**1-inch Burial Depth***					**3-inch Burial Depth**					**5-inch Burial Depth**
	Rep 1	Rep 2	Rep 3	Rep 4	Total	Rep1	Rep 2	Rep 3	Rep 4	Total	Reps 1 - 4
	#	#	#	#	#	#	#	#	#	#	*Total #*
Riverine											
East Yellowstone	24	20	15	10	69	0	0	0	0	0	0
Elk Island	16	19	16	11	62	0	0	0	0	0	0
Roche Jaune	22	23	11	6	62	0	0	0	0	0	0
Seven Sisters	24	20	16	10	70	0	0	0	0	0	0
Upland											
Iverson	21	21	16	11	69	0	0	0	0	0	0
Love Street	22	24	18	6	70	0	0	0	0	0	0
Radar Site	16	20	15	13	64	0	0	0	0	0	0
Westside	24	25	8	9	66	0	0	0	1	1	0
Totals	169	172	115	76	532*	0	0	0	1	1	0

*Significantly higher in emergence than the 3-inch and 5-inch burial depths.

Chart 2. Days to Russian olive seed emergence after planting



There were no statistical differences between the individual riverine and upland populations ($p > 0.2$) in the proportion (distribution) of overall emergence of seed buried at the 1-inch depth. Compared to all the seed sources, Elk Island ($p = 0.03$) and Love Street ($p = 0.001$) were significantly different from the other collection populations for the distribution of live non-emerged, live emerged, and dead non-emerged seed buried 1-inch deep (see Chart 3).

Of the 267 non-emerged seeds from the 1-inch burial depth, 62 (23.2%) were determined to be viable, but had not germinated (see Table 2). There were no viable, non-emerged seed from either the 3- or 5-inch burial depths. It appears as though Russian olive seed in greenhouse environments does not have the lifespan as those in seed storage facilities.

Chart 3. Summary of 1-inch burial results

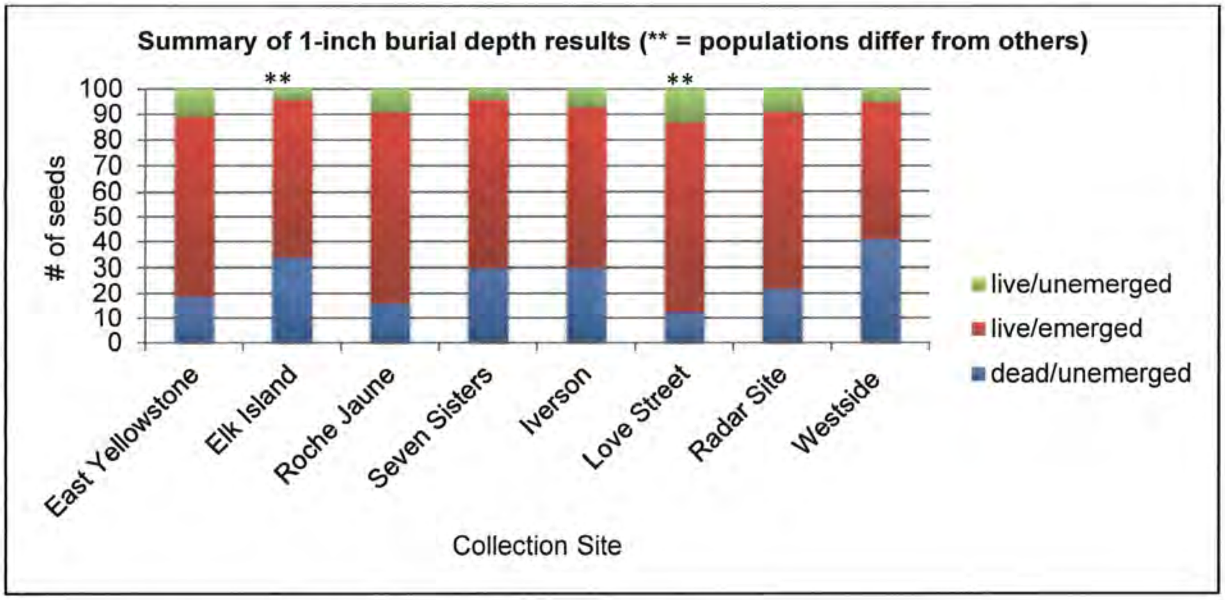


Table 2. Summary of live, non-emerged Russian olive seed in 1-inch burial depth treatment.

Collection Site	**1-inch Burial Depth**				
	Rep1	Rep 2	Rep 3	Rep 4	Totals
	#	#	#	#	#
Riverine					
East Yellowstone	0	0	4	7	11
Elk Island	1	0	2	1	4
Roche Jaune	1	0	3	5	9
Seven Sisters	0	0	1	3	4
Upland					
Iverson	0	0	1	6	7
Love Street	0	1	1	11	13
Radar Site	0	0	2	7	9
Westside	1	0	1	3	5
Totals	3	1	15	43	62

Discussion/Conclusions

Under greenhouse conditions, Russian olive seed has high germination and emergence when planted at a shallow depth (1 inch or less). Of the seeds planted in the study at the 1-inch depth, 89.6 percent germinated and emerged within 30 days of planting. Remaining emergence occurred slowly over the remainder of the study period. A Fort Collins, Colorado nursery planting study, found Russian-olive seed germination occurred over a large time interval when the planting elevation was above the water table and the seeds were exposed to light. In a natural setting, these differences, along with variable cold stratification conditions, may result in germination of Russian olive seeds across multiple growing seasons (Shaforth et al., 1995).

Russian olive does not appear to have the capability of emerging from or surviving burial at or beyond a 3-inch depth under these study conditions. Our results suggest if Russian olive seeds are buried to a 3-inch or greater depth by a flood event, the seed may germinate, but not emerge. Seeds may deteriorate and become non-viable given certain biotic or environmental conditions, such as the presence of predatory insects or pathogens. Insect larvae were observed on the embryo in several of the non-emerged seeds (see Figure 5). Therefore, Russian olive seeds buried beyond 3 inches deep may not be viable if uncovered by a future flood event (see Figure 6). In a study by (Lesica and Miles, 1999) on the Marias River in Montana, Russian olive was not observed on sandbars, but did occur in 89% of low-terrace plots and 47% of high-terrace plots. This, in combination with the results of this study, suggests stream sediment deposition may be one negative factor influencing the emergence of Russian olive seeds within the stream channel.

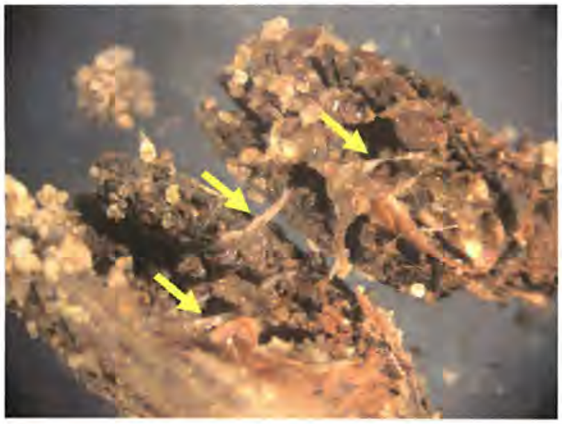


Figure 5. Larvae on a Russian olive embryo

Figure 6. A dead 3-inch depth, germinated seed

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