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TOLERANCE OF BLACK LOCUST (*Robinia pseudoacacia* L.) SEEDLINGS TO PRE APPLIED HERBICIDES

SUMMARY

The field studies were conducted in the nursery of the PE "Macedonian Forests", subsidiary "Karadžica" in Dračevo, Skopje region, during 2014 and 2015 on Fluvisol sandy loam. Tolerance of black locust seedlings to the PRE application of imazethapyr, S-metolachlor, linuron and pendimethalin was studied. The black locust seedlings differed in their response to PRE herbicides. All applied PRE herbicides caused no significant visual injury (< 0.7%) in black locust seedlings in 2014, but linuron and pendimethalin applied in 2015 caused serious black locust seedlings injury which did not decrease over time (48.5% and 60.5% at 28 DAT, and 63.8% and 72.3% at 56 DAT, respectively). The high precipitation which occurred immediately after herbicide application (28 L/m^2) probably was the most likely reason for serious black locust injury caused by these herbicides. PRE application of herbicides in 2014 resulted in statistically similar plant number per m^2, plant height and root collar diameter to the weed-free control. However, all black locust seedlings parameters were significantly affected by linuron and pendimethalin in 2015. Their application resulted in fewer plants per m^2, minor plant height and smaller root collar diameter of black locust seedlings in compare with those in weed-free control.

Keywords: black locust, PRE herbicides, injuries.

INTRODUCTION

Weed management is one of the major production problems for black locust seedling producers and is essential to optimize the yield of this non-competitive crop. Weeds left uncontrolled compete with black locust plants for light, moisture, and nutrients and can drastically reduce black locust quality and yield. In the past the black locust in North Macedonia was planted for reforestation with support of government in areas where local people suffered

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consequences of erosion flows and torrents, than later, refosteration gradually turned into “national reforestation” performed by citizens (Kolevska et. al., 2017). From the tree species, which were grown in forest nurseries in the past, many broadleaf allochtonous species were represented including black locust (Kolevska et. al., 2017).

Effective weed control in black locust nurseries is limited, because no one herbicide is registered for this purpose in North Macedonia. Usually are used herbicides for weed control in *Fabaceae* crops (Pacanoski et al., 2017). Therefore, more research is needed to identify herbicides that provide consistent annual grass and broadleaved weed control and are safe to use on black locust nurseries.

Imazethapyr is an imidazolinone herbicide, which is absorbed by both the roots and shoots. Imazethapyr can effectively control a broad spectrum of weeds such as velvetleaf (*Abutilon theophrasti* Medic.), redroot pigweed (*Amaranthus retoflexus* L.), smartweed (*Polygonum* spp.), lambsquarters (*Chenopodium album* L.), wild mustard (*Sinapis arvensis* L.), common ragweed (*Ambrosia artemisiifolia* L.) and foxtail (*Setaria* spp.) (Bauer et al., 1995; Ward and Weaver, 1996).

S-metolachlor is a chloracetanilide herbicide that is absorbed by germinating grasses through the shoot just above the seed and in broadleaf weeds through the root and shoot. Applications of S-metholachlor can effectively control a number of annual grasses such as foxtail (*Setaria* spp.), large crabgrass (*Digitaria sanguinalis* L. Scop.), barnyardgrass (*Echinochloa crus-galli* L. Beauv.), fall panicum (*Panicum dichotomiflorum* Michx.), and witchgrass (*Panicum capillare* L.) (Osborne et al., 1995; Vencill, 2002). It also provides partial control of some small-seeded broadleaved weeds such as nightshade (*Solanum* spp.), redwood pigweed (*Amaranthus retroflexus* L.), and common lambsquarters (*Chenopodium album* L.) (Senseman, 2007).

Linuron is a substituted urea herbicide registered for use in a number of crops including soybean and green beans (Pacanoski and Glatkova, 2014). Linuron is readily absorbed through roots following a soil application (Senseman, 2007). Linuron applied pre-emergence (PRE) controls many broadleaf weeds such as velvetleaf (*Abutilon theophrasti* Medic.), redwood pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), common ragweed (*Ambrosia artemisiifolia* L.), common chickweed (*Stellaria media* L. Vill.), field pennycress (*Thlaspi arvense* L.), purslane (*Portulaca oleracea* L.), shepherd’s purse (*Capsella bursa-pastoris* L.Medic.), smartweed (*Polygonum* spp.), annual sowthistle (*Sonchus oleraceus* L.) (Pacanoski et al., 2014) and wormseed mustard (*Erysimum cheiranthoides* L.), including acetolactate synthaseand triazine-resistant biotypes (Van Gessel et al., 2000).

Pendimethalin is a dinitroaniline selective herbicide that can control smooth crabgrass (*Digitaria ischaemum* (Schreb) Muhl.), barnyardgrass (*Echinochloa crus galli* L. Beauv.), fall panicum (*Panicum dichotomiflorum* Michx.), large crabgrass (*Digitaria sanguinalis* L. Scop), giant foxtail (*Setaria* *faberii* Herrm.), green foxtail (*Setaria viridis* L. Beauv.), yellow foxtail (*Setaria glauca* L. Beauv.), and certain annual broadleaved weeds such as common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.) (Soltani et al., 2012). Pendimethalin is primarily absorbed by the emerging coleoptile of grasses and hypocotyl/epicotyl of broadleaf weeds (Shaner, 2014).

Tolerance of black locust to various soil applied herbicides should be attributed to application method, herbicide rate, cultivar, environmental and soil conditions. There is currently no registration for use of imazethapyr, S-metolachlor, linuron and pendimethalin in black locust seedling production in North Macedonia, and because of that sensitivity of black locust to these PRE herbicides is not known for North Macedonia growing conditions.

Therefore, the objective of this research was to determine the tolerance of black locust seedlings to imazethapyr, S-metolachlor, pendimethalin and linuron PRE under North Macedonia environmental conditions.

MATERIAL AND METHODS

Field studies were conducted in the nursery of the PE "Macedonian Forests", subsidiary "Karadžica" in Dračevo, Skopje region, during 2014 and 2015 on Fluvisol sandy loam with 10.50% coarse, 63.10% fine sand, 26.40% clay+silt, 3.1% organic matter and pH 7.0. The nursery is located at N41°56.140, E21°30.745, altitude of 250 a.s.l., inclination of 4-5^0, north-west exposition. The experiment method was set at randomized complete block design with four replications, and the size of elementary plot was 15 m^2 (3 x 5m).

Seedbed was prepared by moldboard plowing in the autumn followed by two passes with a field cultivator in the spring. Before seeding in the spring, fertilizer was incorporated at rates indicated by soil tests. One day prior sowing, the black locust seeds were hydro-thermically treated in boiling water for 10 seconds, than cooled in cold water with 10 g Benomil 50 WP/10 kg of seed, and left soaking for 24 hours. Germination of the seed was 65.5%. Black locust seeds were seeded in a well-prepared seedbed at a seeding rate of 25 grams seeds/1 meter of row on May 5th, 2014 and May 14th, 2015, respectively. The interrow spacing was 25 cm and seeding depth was about 2 cm.

Herbicides were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 300 L/ha aqueous solution at 220 kPa. PRE herbicide treatments were applied one day after sowing, on May 6th, 2014 and May 15th, 2015, respectively. PRE herbicide treatments were: imazethapyr (Pivot 100-E) at 1.0 L/ha, S-metolachlor (Dual Gold) at 1.0 kg/ha, linuron, (Linurex 50 SC) at 2.0 L/ha, and pendimethalin (Stomp Aqua) at 5.0 L/ha. Weed-free control, included in the studies, was maintained by 2 hoeing + hand weeding to eliminate the confounding factor of weed interference on black locust seedling crop. Black locust injury was visually evaluated based on a 0% - 100% rating scale, where 0 is no injury to black locust plants, and 100 is complete death of black locust plants (Frans et. al., 1986). The injury was visually rated by determining the average percentage of delayed emergence, hypocotyl swelling, brittle stem at the soil line, plant stunting, chlorosis, or necrosis (or all) occurring in treated black locust plots when compared with nontreated plants. Black locust injury was estimated 28 and 56 days after treatments (DAT). The black locust seedlings of m^2 per every plot were count 56 DAT. 25 plants of black locust seedlings selected per plot, and height from soil surface to the highest point of each plant, as well as root collar diameter were measured 180 DAT, i.e. in the end of black locust vegetation period.

Total monthly rainfalls are shown in Table 1. Generally, 2014 was drier than 2015. Precipitations in May 2014 were very low (20 mm). However, June, and even July were unusually wet months. In August and September precipitation occurred during the three days in the middle of August, and during the first 2 and the last 4 days of September. Opposite, spring of 2015 was humid. Precipitation occurred during May were a little bit above the 30ys average for the Skopje locality; precipitation occurred in the first and at the middle of the second decade of May. Particularly high precipitation occurred immediately after herbicide application (28 L/m^2). In June, precipitation occurred mainly in the second decade of the month (40 L/m^2). Summer months in 2014, particularly July and September, were very humid, 61% above the 30ys average for the Skopje locality (80 mm).

Table 1. Total monthly rainfall from May to October in 2014 and 2015 at the experimental location.

Month	Precipitation (mm)	
	Skopje locality	
	2014	2015
May	20	49
June	51	58
July	48	54
August	10	22
September	23	75

The data were tested for homogeneity of variance and normality of distribution (Ramsey and Schafer, 1997) and were log-transformed as needed to obtain roughly equal variances and better symmetry before ANOVA were performed. Data were transformed back to their original scale for presentation. Means were separated by using LSD test at 5% of probability.

RESULTS AND DISCUSSION

Inconsistent weather patterns between the 2 years of the study likely influenced the crop injury. The humid spring in 2015 (Table 1), particularly high precipitation which occurred immediately after herbicide application (28 L/m^2) probably was the most likely reason for serious black locust injury particularly caused by linuron and pendimethalin estimated at 28 and 56 DAT in 2015 compare with 2014 (Table 2). Because of that, there was a significant treatmentby-year interaction. Visual crop injury symptoms included chlorosis and necrosis of leaves and growth reduction.

Imazethapyr

Imazethapyr applied PRE at 1.0 L/ha caused no significant visual injury in black locust in 2014, but caused 7.8% injury at 28 DAT and 4.3% injury 56 DAT in 2015 (Table 2). Furthermore, Şarpe et. al., (20011), reported that black locust seedlings in the 1st year of vegetation tolerated very well the imzaethapyr. With the exception of root collar diameter in 2015, there were no significant differences among black locust seedlings parameters when imazethapyr was applied in both years compared to the weed-free control (Table 3). Similar results were reported by Soltani et al., (2015). Imazethapyr applied PRE caused no significant visual injury in adzuki bean at 75 g a.i./ha, but caused 4% injury at 14 DAE and 5% injury 28 DAE at 150 g a.i./ha in adzuki bean. No adverse effect on plant height, shoot dry weight, seed moisture content and yield of adzuki bean was found with 75 g a.i./ha and 150 g a.i./ha. Also, and other studies with Phaseolus spp. have shown that imazethapyr applied PRE can cause up to 6% visual injury in black bean (Soltani et al., 2004a).

S-metolachlor

S-metolachlor applied PRE at 1.0 kg/ha resulted in 0.4 and 0.3% visual crop injury in black locust 28 and 56 DAT, respectively in 2014. The same herbicide caused 10.3% visual injury 28 DAT, and injury did decrease over time in 2015 (Table 2). Plants per m^2, plant height and root collar diameter were not affected by application with S-metolachlor with the exception of plant height and root collar diameter in 2015. For example, S-metolachlor application resulted in more plants per m^2, greater plant height and bigger root collar diameter of black locust plants in 2014 compared to the weed-free control (Table 3). Similarly, the PRE application of S-metolachlor at 1.6 kg/ha resulted in less than 8.3% visual crop injury in black beans, and did not cause any significant plant height or dry weight reduction in black beans (Soltani et al., 2004a). Dry bean tolerance to S-metolachlor was acceptable in other research (Soltani et al., 2003; Soltani et al., 2004b; Sikkema et al., 2004). Opposite, S-metolachlor at 1600 g/ha caused 21% visual injury 7 DAE, and decreased plant height. However, shoot dry weight, seed moisture content, and yield of adzuki bean were not reduced (Sikkema et al., 2006).

Linuron

At 28 and 56 DAT in 2014, linuron caused 0.7 and 0.4% black locust seedlings injury, respectively. But, in 2015 linuron caused serious black locust seedlings injury (48.5% at 28 DAT, and 72.3% at 56 DAT, respectively) which did not decrease over time (Table 2). Injury increased in 2015, because Skopje region received 29 mm more precipitation in May compared to the same month in 2014. It is likely that these precipitations which mainly occurred 18 to 20 hours after linuron application contributed to serious black locust injury. Linuron applied at 2.0 L/ha in 2014 resulted in statistically similar plant number per m^2, plant height and root collar diameter to the weed-free control. However, linuron application in 2015 significantly reduced plant number per m^2, plant height and root collar diameter. There were 393 plants per m^2 in weed-free control compared to significantly lower number of plants per m^2 of 228 in plots treated with linuron. Black locust seedling plants were almost 30 cm lower and more than 25 mm thinner in compare with those in weed-free control (Table 3). It is reported that seeds of black locust in greenhouse condition are sensitive to most of pre-emergence herbicides, including linuron (Geyer and Long, 1991). In investigations of Pacanoski and Glatkova (2014) linuron caused 13.8% of green beans injury because of a heavy rainfall shortly after their emergence. Linuron applied PRE caused as much as 12% injury in cranberry and kidney bean, 47% injury in black bean, and 56% injury in white bean. Linuron had no effect on the height of cranberry and kidney bean, but decreased the height by 7, 8, and 15% in black bean and by 10, 13, and 23% in white bean at 1500, 2000, and 2500 g ai/ha, respectively (Sikkema et al., 2009). The greater mobility of linuron might be related to its higher water solubility (64 mg x L^{-1}) and smaller adsorption coefficient (Koc of 400 L x kg^{-1}) (El Imache et al., 2008). Because of that linuron leaching, and thus its potential to injury black locust seedlings is possible, particularly when heavy rainfall follows its application.

Table 2 Visual crop injury (%) of black locust seedlings treated with PRE herbicides at Skopje region, North Macedonia, in 2014 and 2015[a-c].

	Visual crop injury (%)				
		28 DAT		56 DAT	
Treatments	Rate (L;kg/ha)	2014	2015	2014	2015
Weed-free control	-------	0.0[b]	0.0[d]	0.0[c]	0.0[b]
Imazethapyr	1.0	0.5[ab]	7.8[cd]	0.3[ab]	4.3[b]
S-metolachlor	1.0	0.4[ab]	10.3[c]	0.3[ab]	6.1[b]
Linuron	2.0	0.7[a]	60.5[a]	0.4[a]	72.3[a]
Pendimethalin	5.0	0.3[ab]	48.5[b]	0.1[bc]	63.8[a]
LSD 0.05		0.69	8.38	0.22	10.50
Random effect interaction PRE herbicides x year		*		*	

[a]Abbreviation: PRE-preemergence; *Significant at the 5% level according to a Fisher's protected LSD test at P<0.05.

[b]Black locust injury was estimated 28 and 56 DAT.

[c]Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05

Pendimethalin

There was minimal injury in seedlings of black locust with pendimethalin applied PRE at 5.0 L/ha estimated 28 and 56 DAT in 2014. However, pendimethalin applied in 2015 caused 48.5 and 63.8% black locust seedlings injury 28 and 56 DAT, respectively (Table 1). The nursery in 2015 received more rainfall immediately after pendimethalin application, which may explain why injury caused by this herbicide was so severe at this year. Additionally, among the dinitroanaline herbicides, pendimethalin has greater water solubility of 0.275 *u*g mL^{-1} (Senseman, 2007). However, the research of Şarpe et. al., (20011), showed that black locust seedlings in the 1st year of vegetation tolerated very well the herbicide pendimethalin. The application of pendimethalin in 2014 resulted in similar plant number per m^2 and plant height compared to the weed-free control, but 2 mm bigger root collar diameter, which was also statistically similar to the weed-free control. However, all black locust seedlings parameters were significantly affected by pendimethalin in 2015. For example, pendimethalin application resulted in fewer plants per m^2, minor plant height and smaller root collar diameter of black locust seedlings (Table 3).

Table 3. Plants number per m^2, plant height (cm) and root collar diameter (mm) of black locust seedlings treated with PRE herbicides at Skopje region, North Macedonia, in 2014 and 2015[a-c].

Treatments		Black locust plants per m^2		Root collar diameter (mm)		Plant height (cm)	
	Rate (L;kg/ha)	2014	2015	2014	2015	2014	2015
Weed-free control	-------	373^a	393^a	45^a	51^a	5.0^{ab}	5.6^a
Imazethapyr	1.0	365^a	401^a	43^a	46^{ab}	4.6^b	4.6^b
S-metolachlor	1.0	389^a	388^a	47^a	42^b	5.3^a	4.4^b
Linuron	2.0	353^a	228^c	42^a	23^c	5.0^{ab}	3.0^c
Pendimethalin	5.0	378^a	275^b	43^a	27^c	5.2^{ab}	3.3^c
LSD 0.05		50.47	26.53	5.78	6.32	0.66	0.84
Random effect interaction PRE herbicides x year		*		*		*	

[a]Abbreviation: PRE-preemergence; *Significant at the 5% level according to a Fisher's protected LSD test at P<0.05.
[b]Plants number per m^2 were measured 56 DAT, plant height and root collar diameter were measured 180 DAT
[c]Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05

Application of pendimethalin has injured both foliage and roots of certain nursery crops, including azalea (*Rhododendron* spp.), Japanese holly (*Ilex crenata* Thunb.) and ornamental grasses (Derr and Simmons 2006). Pendimethalin application in combination with excessive moisture (rainfall or irrigation) can result in injury to seedling cotton (Grey and Webster, 2013). Opposite, Soltani et al., (2013) concluded minimal injury in various market classes of dry bean with pendimethalin applied PPI or PRE at 1080 or 2160 g ai/ha one and two Weeks After Emergence (WAE). However, pendimethalin applied PRE caused slightly greater injury than pendimethalin applied PPI at 4 WAE.

CONCLUSIONS

In most countries the effective weed control in black locust nurseries is quite difficult, because there are few registered herbicides or none for this purpose. The PRE application of herbicides in 2014 resulted in statistically similar plant number, plant height and root collar diameter to the weed-free control. Contrary, in 2015 the all black locust seedlings parameters number of plants, minor plant height and smaller root collar diameter were significantly affected by linuron and pendimethalin in compare with those in weed-free control.

However, the application of PRE herbicides for weed control for production of black locust seedlings in future should be based on soil type and particularly on amount of rainfall immediately after herbicide application. The results showed that most of used herbicides due to amount of the precipitation caused injury to the black locust, so in the future the use of pre-emergence herbicides to combat weeds in black locust should be based on the monitoring of climatic conditions and especially when we have inadvertently the fact of climate change in recent times. These conclusions are based on certain area and small-scale field experiment, and underestimate the results of herbicides achieved in these climatic conditions, certainly in the future similar research should be conducted in other areas of the country.

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