Auburn University
Southern Forest Nursery
Management Cooperative

RESEARCH REPORT 12-05

EFFECTS ON HERBICIDE GALL FORMATION AND SURVIVAL FROM APPLICATIONS OF UNDILUTED PENDULUM® AQUACAP ™ TO LOBLOLLY PINE SEEDLINGS

by
D. Paul Jackson and Barry Brooks

INTRODUCTION

Pendulum® AquaCap™ (PAC) (pendimethalin) is a dinitroaniline herbicide registered for use in forest tree nurseries that provides good prostrate spurge (Chamaesyce maculate) control. However, in some of our herbicide trials PAC has caused herbicide galls to form at the ground-line on loblolly pine (Pinus taeda) stems with postemergence applications in the nursery (South and Hill 2009; Jackson and South 2012). Similarly, postemergence applications of pendimethalin to field-grown cotton (Gossypium hirsutum) caused injury that was described as "an enlarged growth of the cotton stem for 7 to 10 cm at the soil surface" (Miller and Carter 1980). Those authors speculated that the cotton injury was associated with increased temperatures caused by soil covering the herbicide-treated portion of the stem during the hotter months of June and July. In a subsequent trial, Miller and Carter (1980) painted the stems of similar aged field-grown cotton plants with undiluted pendimethalin and were unsuccessful in creating the injury in the absence of soil covering the treated area.

Because the pendimethalin injury on pine seedlings is similar to that reported with cotton (Miller and Crater 1980), we have speculated that factors such as heat and soil texture may also contribute to gall formation when PAC is applied after pine germination (South and Hill 2009). However, not all of the PAC herbicide galls on pine seedlings have formed at the ground-line. In a trial where PAC applications were made 2 weeks after sowing, galls formed higher up on the stem near the cotyledon scar (Jackson and South 2012). This was the first time PAC galls formed on a different part of the seedling other than on the stem near the treated area of soil.

Like Miller and Carter's "painting pendimethalin trial", we wanted to determine if herbicide injury could be induced using direct applications of undiluted PAC to different parts of pine seedlings. Therefore, the objectives of this trial was to 1) evaluate seedlings for herbicide gall formation after direct applications of undiluted Pendulum® AquaCap™ to the stem, foliage, or

container root plug of loblolly pine seedlings, and 2) determine if the presence of galls on seedlings affects their survival after outplanting.

METHODOLOGY

Seeds from a half-sib family of loblolly pine were stratified for 40 days and then sown into Hiko™ V-93 containers with a volume of 93 cm^3 (5.7 in^3) and seedling density of 526 m^2 (49 ft^2) on March 22, 2011. The growing media was composed of 70-80% Canadian sphagnum peat moss, coarse grade vermiculite, and perlite. After sowing, the seeds were covered with a thin layer of coarse perlite and containers were placed in a completely randomized block design on a greenhouse table.

Seven herbicide treatments were applied to seedlings on May 16, 2011 using undiluted Pendulum® AquaCap™ (Table 1). Twenty cavities were sown with loblolly pine per container set and each set was an experimental unit with four container sets (replications) per treatment. For the root plug treatment, three drops of PAC was placed on top of the media without touching any seedling tissue above the media surface (Figure 1A). Each drop delivered approximately 1 ml of PAC with a total of 3 ml applied to the root plugs. The amount of PAC applied to seedling stems and foliage was not quantified, however, the methods used to apply PAC were consistent for every seedling application. A small-bristled paintbrush was used to apply PAC to the foliage and stem (Figure 1B and 1C). The brush was dipped into the PAC one time and the stem or foliage received three strokes from the brush. The brush was rinsed in a beaker of water, patted dry, and wiped clean with a paper towel between each application. To prevent washing away the herbicide from seedling stems and foliage, the seedlings were irrigated by placing the container sets in a tub of water and allowing the water to absorb up the root plug. Fertilization took place as needed with Miracle Gro® (azalea/camellia/rhododendron) 30-10-10 [$30N:4P_2O_5:8K_2O$] water-soluble fertilizer by the method of placing container sets in a tub of fertilizer/water solution.

On August 31, 2011, root collar diameter (RCD), height, and survival were measured on the root plug-treated seedlings. Due to the high seedling mortality in the root plug treatment, seedling root quality was assessed on living seedlings by destructively analyzing the root systems for root number, root length, root surface area, and root diameter using a WinRhizo™ scanner and computer software (Regent Instruments, Inc., Quebec City, Quebec, Canada). A sample of non-treated control seedling roots were also scanned and analyzed as a comparison to the root plug treatment. Root morphology was not measured on seedlings that received foliage and stem herbicide treatments as they were to be outplanted to determine survival.

On October 5, 2011, seedlings from each of the two foliage and stem herbicide treatments and non-treated controls were measured for RCD, height, and survival. Afterwards, the remaining seedlings were outplanted in the Nursery Cooperative's seedling stress facility in a completely randomized block design. In this trial, 4 boxes (4'x4'x3') were used that contained coarse sand and that were protected from rainfall and shaded by a roof. Each box was considered a replication. The seedlings in the boxes were watered twice per week until February 1, 2012. To induce drought stress and allow treatment effects to bear out, seedlings were not watered again until May 1, 2012. From that point, watering took place twice per week, and seedling RCD,

height, and survival was measured again on July 2, 2012. Data was analyzed in SAS using a general linearized model (GLM), and the least significance difference (LSD) is reported to reveal the statistical power of the analyses.

RESULTS AND DISCUSSION

Greenhouse phase. After 6 months of greenhouse culture, all of the seedlings that received the root plug and stem treatments at both 8 and 8+10 weeks post-sowing developed stem galls. The galls ranged in severity, size, and shape. In most cases, the galls tended to be elongated or spindle-shaped along the stem that mirrored the area treated with PAC using the paintbrush (Figure 2A). Other galls were more rounded and localized (Figure 2B), while some developed lesions (Figure 2C). The type of gall that occurred did not follow a treatment pattern. The foliage treatments at either 8 or 8+10 weeks post-sowing did not cause stem swellings. This is the first report of herbicide galls forming on seedling stems after direct applications of undiluted Pendulum® AquaCap™ to the container root plug without the herbicide touching the seedling. Miller and Carter (1980) is the only other known trial where undiluted pendimethalin was applied to plants, but they were unable to induce an herbicide injury.

Seedling root growth was affected by applications of PAC to the root plug and the effects were more severe when PAC was applied twice to seedlings. Root length, root surface area, root diameter, and the number of roots were all significantly lower when PAC was applied to the root plug (Table 2). Non-treated control seedlings had 3x more root tips (224) than those treated 8 weeks post-sowing (73) and about 4x more root tips (58) than seedlings treated at 8+10 weeks post-sowing. Seedling survival was 38% for seedlings that received PAC on the root plug at both 8 and 8+10 weeks post-sowing, which was significantly lower than the seedling survival for the stem and foliage treatments (Table 3).

Pendimethalin's mode of action involves the absorption of the herbicide into newly formed root tissue. Once PAC is absorbed into plant tissue, the process of cell division is disrupted, and developing root cells can then no longer divide and are rendered, distorted, swollen, and non-functional. After applying PAC to the root plug, the herbicide likely moved down the plug and settled in the media around the developing root system, where it was absorbed by new fine feeder roots. This may explain the negative effects on root morphology, the steady reduction in seedling vigor, and increased mortality for seedlings in the PAC root plug treatment.

The foliage treatment applied 8 weeks post-sowing and all three PAC treatments applied at 8+10 weeks post-sowing had significantly larger root collar diameters than non-treated control seedlings (Table 4). As expected, the seedlings with stem galls had larger RCDs, but both the 8 and 8+10 week post-sowing foliage treatments did not have stem galls. The foliage treatment at 8 weeks post-sowing also produced taller seedlings than non-treated seedlings (Table 5). Seedling heights for both root plug treatments were significantly shorter than all other treatments, and the reduction in height at 8 and 8+10 week post-sowing was about 5 cm and 6.5 cm shorter than non-treated seedlings, respectively.

Outplanting phase. The focus of the outplanting phase was to determine how well seedlings with herbicide galls (stem treatments) could survive after outplanting compared to seedlings

without herbicide galls (foliage treatments and non-treated controls). Because seedlings were destructively sampled for root system analysis, the PAC root plug treatments were not outplanted. Seedlings remained outplanted from October 2011 to July 2012 and without irrigation from February1 to May 1. Despite three months of drought, only three seedlings died in the outplanting trial. The herbicide galls did not affect seedling survival with both the 8 and 8+10 week post-sowing stem treatments having 100% survival (Table 3).

This was the first herbicide gall versus no herbicide gall survival trial conducted by the Nursery Cooperative using container-grown seedlings. Another Nursery Cooperative outplanting trial using bareroot loblolly pine resulted in seedlings having significantly higher survival without galls (45%) compared to those with PAC galls (24%) (unpublished data). However, the seedlings in that trial were outplanted in March, which may have confounded results as environmental conditions during March favor declines in survival compared to outplanting conditions from December to February (Wakeley 1954; Venator 1985). In contrast, a bareroot seedling survival trial comparing seedlings with and without galls caused by the herbicide Barricade® (prodiamine) showed no difference in survival when outplanted in January (Carey 2000).

Nine months after outplanting, non-treated control seedlings had significantly smaller RCDs than all other treatments with the exception of the foliage treatment applied at 8+10 weeks post-sowing (Table 4). On average, seedling RCD for the stem treatments grew 0.7 mm more than the foliage treatments after outplanting, which was due to the presence of stem galls that resulted in larger RCDs. Conversely, seedlings that received foliage treatments were significantly taller than all other treatments, and on average, grew about 2 cm more than non-treated seedlings (Table 5).

CONCLUSIONS

In this trial, we applied undiluted Pendulum® AquaCap™ directly to specific areas of container-grown loblolly pine seedlings to determine if herbicide galls were inducible, and if so, how galled seedlings survived after outplanting. Obviously, the herbicide is normally applied in a dilution with water, so the methods used in the trial are not operational. The negative effects on root growth and subsequent seedling mortality from the PAC root plug treatments can be explained by the herbicide's mode of action, which deforms developing roots and renders them non-functional. One thing that cannot be explained is the formation of stem galls after applying PAC directly to the root plug. At no time did any portion of the seedling above the media receive the herbicide.

With respect to the reports of herbicide gall formation when PAC is used postemergence to seedlings in the nursery, the following question needs to be answered: "If herbicide galls develop on seedling stems as a result of Pendulum® AquaCap™ applications, will they survive after outplanting?" In these trials, the presence of herbicide galls did not affect seedling survival nine months after outplanting. During that time, the seedlings endured three months of drought, but avoided the stress of direct sunlight and increased temperatures by being under a shadehouse. Conversely, an unpublished Nursery Cooperative outplanting trial demonstrated that bareroot seedling survival was significantly better without galls present compared to seedlings with galls. However, the results may have been confounded by the seedlings being outplanted in the warmer and drier month of March. While these results are promising with respects to seedling survival,

an outplanting trial is needed to evaluate the survival of bareroot seedlings with and without PAC herbicide galls when outplanted in a normal planting window (December-February) and under normal environmental conditions. While these results are encouraging for seedling survival, if PAC is used to control spurge, it is recommended that the herbicide be applied preemergence to seedlings at the time of sowing.

REFERENCES

Carey, B. 2000. Survival and growth of loblolly pine seedlings with or without Prodiamine caused basal galls did not differ after one year in the field. Southern Forest Nursery Management Cooperative, School of Forestry and Wildlife Sciences, Auburn University. Spring Newsletter.

Jackson, D.P. and D.B. South. 2012. Screening of Pendulum® AquaCap™ on Tower® herbicides on pine seedbeds. Southern Forest Nursery Management Cooperative, School of Forestry and Wildlife Sciences, Auburn University. Research Report 12-01: 14 p.

Miller, J.H. and C.H. Carter. 1980. Performance of six substituted dinitrobenzamine herbicides applied at layby of cotton (*Gossypium hirsutum*). Weed Science 28(2): 212-215.

South, D.B. and T.E. Hill. 2009. Results from six *Pinus taeda* nursery trials with the herbicide pendimethalin in the USA. Southern Forests 71(3): 179-185.

Venator, C.R. 1985. Survival of shortleaf pine (*Pinus echinata* Mill.) seedlings as influenced by nursery handling and storage. Tree Planters' Notes 36(4): 17-19.

Wakeley, P.C. 1954. Planting the southern pines. USDA Agricultural Monograph, No. 18: 233 p.

Table 1. Treatment site with Pendulum® AquaCap™, the timing of herbicide treatment, and the method used to apply the herbicide on March 22, 2011.

Herbicide Treatment Site	Treatment* (weeks post-sowing)	Application Method
Non-treated control	--------	Did not receive herbicide.
Root plug	8	Applied with a dropper; no herbicide on seedling above media.
Foliage	8	Painted with brush.
Stem	8	Painted with brush.
Root plug	8+10	Applied with a dropper; no herbicide on seedling above media.
Foliage	8+10	Painted with brush.
Stem	8+10	Painted with brush.

* 8 = treatment applied 8 weeks post-sowing; 8+10 = treatment applied at both 8 and 10 weeks post-sowing

Table 2. Mean root length, root surface area, root diameter, and number of root tips for loblolly pine seedlings with Pendulum® AquaCap™ applied only to the root plug. Data was recorded 6 months after sowing on August 31, 2011.

Herbicide Treatment Site	N	Treatment* (weeks post-sowing)	Root Length (cm)	Root Surface area (cm^2)	Root Diameter (mm)	Root tips (#)
Non-treated control	20	-------	124.6	56.1	1.49	224.2
Root plug	25	8	52.1	20.4	1.17	73.2
Root plug	29	8+10	47.7	16.2	1.06	58.6
LSD**			*14.0*	*5.6*	*0.18*	*25.9*

* 8 = treatment applied 8 weeks post-sowing; 8+10 = treatment applied at both 8 and 10 weeks post-sowing
** Least significant differences are italicized.

Table 3. Mean survival for loblolly pine seedlings treated with Pendulum® AquaCap™ after 6 months of greenhouse culture on August 31, 2011 (Survival #1) and 9 months after outplanting on July 2, 2012 (Survival #2).

Herbicide Treatment Site	Treatment* (weeks post-sowing)	Survival #1 (%)	Survival #2 (%)
Non-treated control	--------	100	98
Root plug	8	38	-------
Foliage	8	100	99
Stem	8	96	100
Root plug	8+10	38	-------
Foliage	8+10	96	99
Stem	8+10	89	100

* 8 = treatment applied 8 weeks post-sowing; 8+10 = treatment applied at both 8 and 10 weeks post-sowing

Table 4. Mean root collar diameter (RCD) for loblolly pine seedlings treated with Pendulum® AquaCap™ after 6 months of greenhouse culture on August 31, 2011 (RCD #1) and 9 months after outplanting on July 2, 2012 (RCD #2).

Herbicide Treatment Site	Treatment* (weeks post-sowing)	RCD #1 (mm)	RCD #2 (mm)	RCD growth (mm)
Non-treated control	--------	2.59	3.21	0.45
Root plug	8	3.01	-------	-------
Foliage	8	3.54	3.63	0.09
Stem	8	3.09	3.90	0.81
Root plug	8+10	3.37	-------	-------
Foliage	8+10	3.27	3.40	0.12
Stem	8+10	3.17	3.87	0.71
LSD**		*0.58*	*0.20*	*0.55*

* 8 = treatment applied 8 weeks post-sowing; 8+10 = treatment applied at both 8 and 10 weeks post-sowing
** Least significant differences are italicized.

Table 5. Mean height for loblolly pine seedlings treated with Pendulum® AquaCap™ after 6 months of greenhouse culture on August 31, 2011 (Height #1) and 9 months after outplanting on July 2, 2012 (Height #2).

Herbicide Treatment Site	**Treatment* (weeks post-sowing)**	**Height #1 (cm)**	**Height #2 (cm)**	**Height growth (cm)**
Non-treated control	--------	18.8	22.7	4.0
Root plug	8	13.9	-------	-------
Foliage	8	20.7	26.8	6.1
Stem	8	18.3	23.1	4.9
Root plug	8+10	12.4	-------	-------
Foliage	8+10	18.3	23.5	5.2
Stem	8+10	16.9	21.3	4.5
LSD**		*1.1*	*1.4*	*1.2*

* 8 = treatment applied 8 weeks post-sowing; 8+10 = treatment applied at both 8 and 10 weeks post-sowing
** Least significant differences are italicized.

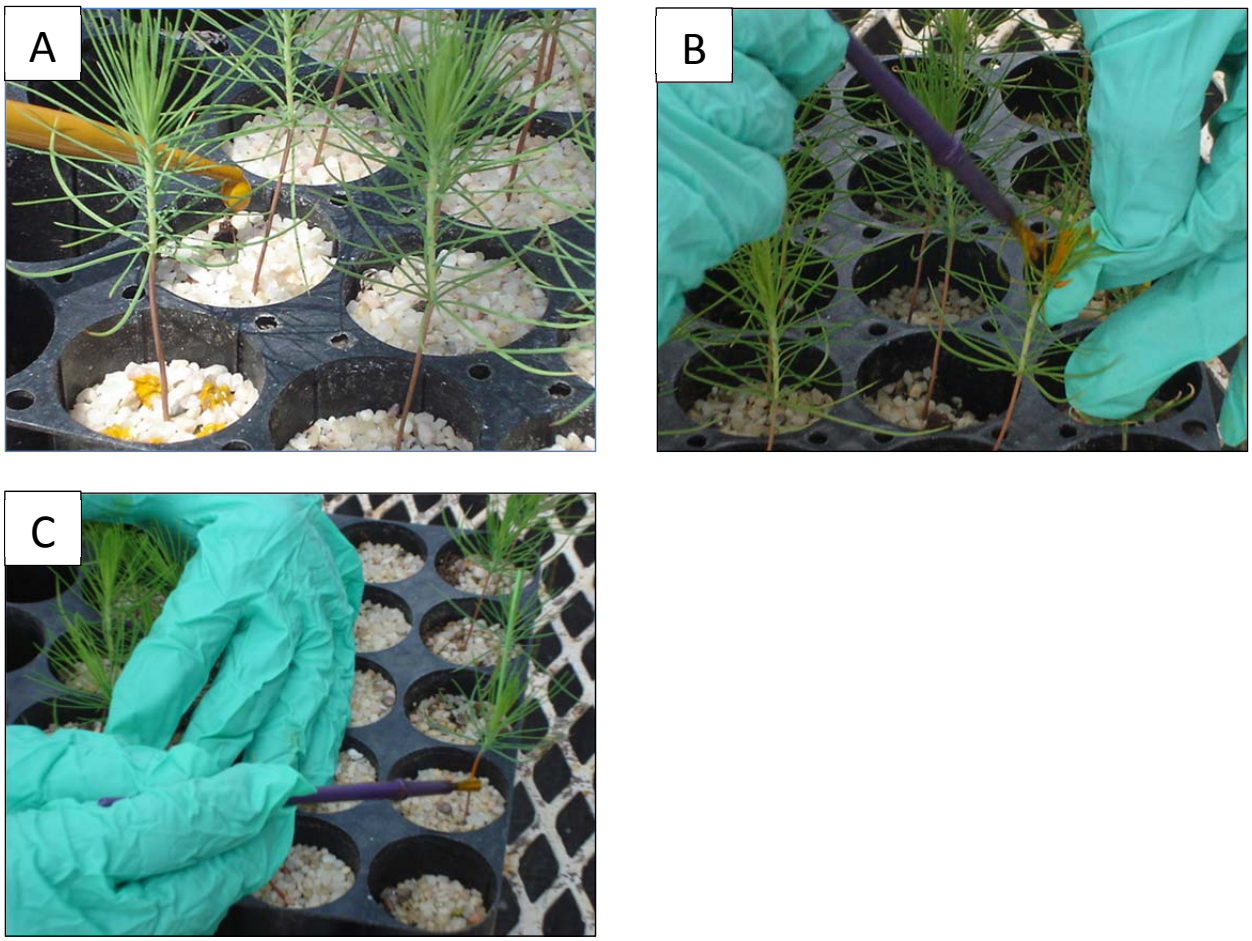

Figure 1. Method used to apply the Pendulum® AquaCap™ treatments to loblolly pine seedlings: A) a dropper was used on the root plug, B) a brush to the foliage, and C) a brush to the stem.

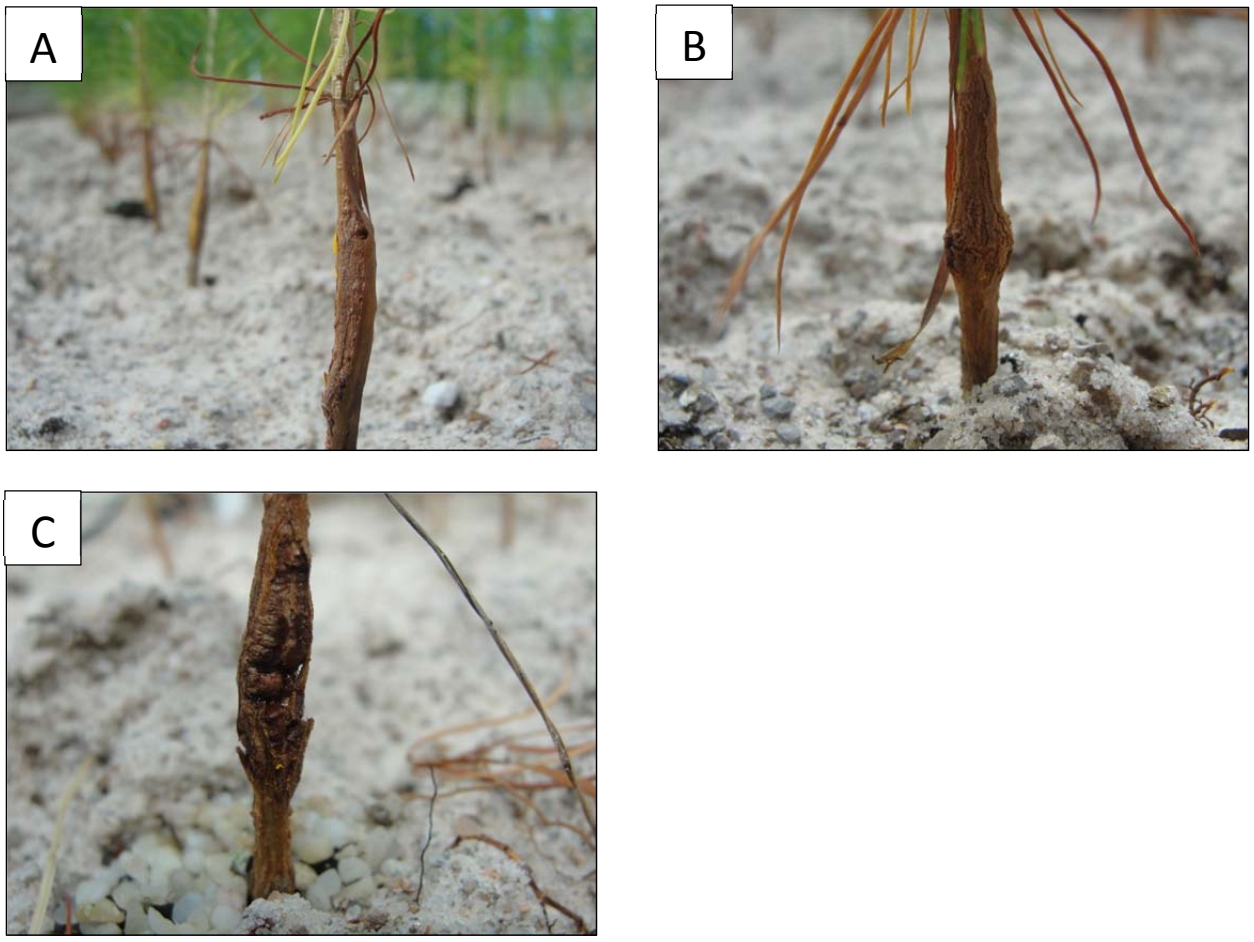

Figure 2. The types of swellings that occurred on loblolly pine seedlings from the root plug and stem Pendulum® AquaCap™ treatments after 6 months of greenhouse culture: A) an elongated swelling up the stem, B) a more rounded and localized gall, and C) an elongated swelling with severe lesion.