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Full Length Research Paper

Gravitropism of loblolly pine (*Pinus taeda*) radicles after chemical sterilization of seeds

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Certain types of chemicals can affect the gravitropism of roots. In a laboratory study, intact loblolly pine (*Pinus taeda* L.) radicles (emerged from H_2O_2- treated seeds) exhibited positive gravitropism 8 h after horizontal placement in sterile conditions. The growth angle decreased from almost horizontal (85°) to 21° within one week after treatment (90° is horizontal and 0° is vertical). When seeds were treated with $HgCl_2$, radicles under sterile growing conditions expressed gravitropism 6.9 h after horizontal alignment. Growth angle changed from 91° to 64° over a 10-day period. Cubic and quadratic functions were used to model growth angle as a function of time. Under similar experimental conditions, radicles from $HgCl_2$-treated seeds showed a greater degree of gravitropism than those from H_2O_2-treated seeds (as indicated by the growth angle). These results indicate that the gravitropism can occur in sterile environments and that the type of chemicals used to sterilize seeds might affect the rate of geotropic response.

Key words: Loblolly pine, radicle, geotropic growth, microorganism, H_2O_2, $HgCl_2$, seed sterilization.

INTRODUCTION

Certain chemicals can affect root growth of plants and some will alter root gravitropism. Chlorsulfuron and met-sulfuron-methyl caused severe ultrastructural alterations and injuries of root caps in pea (*Pisum sativum* L.) and maize (*Zea mays* L.), and affected root gravitropism (Fayez et al., 1995). Tetrazolium altered gravitropism of primary roots of barley (Hordeum vulgare L.), oat (*Avena sativa* L.), rape (*Brassica napus* L.), sheep fescue (*Festuca ovina* L.), and wheat (T*riticum aestivum* L.) (Steiner and Fuchs, 1987). Hydrogen peroxide (H_2O_2) and mercuric chloride ($HgCl_2$) are sometimes used to decontaminate the surface of seeds (Gyimah, 1977; Somade, 1998; Sharma et al., 2004), but it is not known if these chemicals affect gravitropism of conifer radicles. Soaking Eucalyptus seeds in H_2O_2 can sometimes dam-age radicles and can result in abnormal germination (Donald and Lundquist, 1988).

When treating pine seeds, concentrations of H_2O_2 typically ranged from 3% to 30% and exposure times varied from 0.25 to 48 h (Barnett, 1976; Mason and Arsdel, 1978; Penafiel, 1982; Graham and Linderman, 1983). Recommended treatment times for loblolly, longleaf (*Pinus palustris* Mill.), shortleaf (*Pinus echinata* Mill.), and slash (*Pinus elliottii* Engelm.) pine varied by species (0.5, 1, 1, 0.25, and 1 h, respectively)(Barnett, 1976). $HgCl_2$ has been used to treat a wide range of seeds including groundnut (*Arachis hypogaea* L.), maize, rice (*Oryza sativa* L.), sorghum [*Sorghum bicolor* (L.) Moench.], soybean [*Glycine max* (L.) Merr.], sunflower (*Helianthus annuus* L.), and wheat (Sweet and Bolton, 1979) . Gilmour and Vanner (1973) pointed out that $HgCl_2$ could be used as a fungicide on Monterey pine (*Pinus radiata* D. Don) seedlings. However, only a few research-ers have sterilized pine seeds with $HgCl_2$ (Tang, 2000; Han et al., 2003). In one study, some loblolly pine radicles (emerged from seeds that had been treated with $HgCl_2$) did not exhibit gravitropism (personal communication:

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Abbreviations: h= hour; min= minute; s=standard deviation

Experimental conditions involving gravitopism of loblolly pine radicles in sterilized Petri dishes in 2001.

Test	Chemical	Temperature	Conditions	Start date	End date
I	H_2O_2	24.6°C, measured at 08:00 and 17:00	Under a fluorescent lamp on the lab table	Jun 18	Jul 16
II	H_2O_2	30°C, and then 25.5°C, measured at 17:00	Incubator (constant light) first, and then under a fluorescent lamp on the lab table	Jul 9	Jul 31
III	$HgCl_2$	30°C	Incubator (constant light)	Aug 22	Sep 19
IV	H_2O_2 $HgCl_2$	30°C, and then 22.7°C, measured at 17:00	Incubator (constant light) first, and then under a fluorescent lamp on the lab table	Aug 31	Oct 11
V	H_2O_2 $HgCl_2$	27°C	Incubator (constant light)	Sep 18	Oct 20

Dr. Walt Kelley, Auburn University).

To date, there are no reports demonstrating that gravitropism of pine radicals are affected by chemical sterilization treatments. Therefore, the objectives of this study were: (1) to test the null hypothesis that after chemical treatment, young radicles of loblolly pine do not express gravitropism under sterile conditions, and (2) the expression of gravitropism is the same for both $HgCl_2$ and H_2O_2.

MATERIALS AND METHODS

All seeds were sterilized with either H_2O_2 or $HgCl_2$ (Table 1). Unsterilized seeds were not used for comparison since growth of microorganisms in dextrose agar can hinder root orientation and can interfere with measurement of the growth angle. Trials were carried out in the Southern Forest Nursery Management Cooperative Lab and Forest Biology Lab, School of Forestry and Wildlife Sciences, Auburn University.

Experiment I

Loblolly pine seeds were sterilized in 9% H_2O_2 for 50-60 min, and rinsed twice for 5 min in sterile distilled water. Six seeds were placed on each plastic dish (100×15-mm)(Fisher Scientific Co., LLC, Suwanee, GA 30024) containing potato dextrose agar (DIFCO Laboratories, Detroit, MI 48232- 7058). Thirty-three petri dishes (each containing 6 pine seeds) were placed under a fluorescent lamp at 24.6°C. After 10 days, 8 petri dishes with no microbial contamination were selected. Seeds with radicles approximately 5 mm long were re-arranged so that 3 seeds per dish had radicles pointing down. Gravity stimulus was introduced when radicles reached 1-1.5 cm long. Each dish was placed on its edge so the gravity stimulus was perpendicular to the growth direction of the radicle tip. Using a lamp and a piece of plotting paper, measurements were made on the position of the growing radicle point. Coordinates were recorded every 2 h for the first 24 h after initiation of gravity stimulus and daily for another 5 days.

Experiment II

Pine seeds were sterilized as in Experiment I. Eighteen petri dishes (each containing 6-7 loblolly pine seeds) were placed under a constant light regime in a Low Temperature Illuminated Incubator (Model 818 of Precision, Winchester, VA) set for 30°C. After 10 days, 6 dishes with no microbial contamination were selected and 3-4 germinated seeds per dish were re-arranged. Growing conditions were similar to that described as in Experiment I (placed under a fluorescent lamp at 25.5°C). Observations were made every 4 h in the first 28 h, twice a day for the following 4 days, and then once a day for additional 7 days.

Experiment III

Pine seeds were sterilized in 0.1% $HgCl_2$ for 3.5 min and rinsed twice for 5 min. Treated seeds were placed in 100x15-mm plastic petri dishes containing potato dextrose agar. Thirty sterilized seeds were placed in three 100x15-mm plastic petri dishes (10 seeds per dish) containing potato dextrose agar under a continuous temperature regime (30°C) with light in the Low Temperature Illuminated Incubator. Observations for microorganism colonies and germination, and measurements on the position of the growing radicle point were made twice a day during the first 5 days, and then daily until 28 days after treatment.

Experiments IV and V

The same procedure was employed in both Experiments IV and V. Fifty seeds were sterilized in 0.1% $HgCl_2$ for 3.5 min and 50 seeds in 9% H_2O_2 for 60 min, respectively, and both rinsed twice for 5 min. $HgCl_2$-treated seeds and H2O2-treated seeds were placed in 12 dishes per treatment (8-9 seeds per dish). Dishes were placed into an incubator at 30°C and 27°C for Experiments IV and V, respectively. Clean seeds were transferred to a new dish. At the start of the trial, 4- 6 clean germinated seeds per dish were re-arranged. The petri dish was rotated 90° clockwise as soon as radicles reached 2.7 cm (s=1.55 cm) and 4.2 cm (s=0.81 cm) for Experiments IV and V, respectively. Dishes in Experiment IV were placed on their edges on a lab table at room temperature (22.7°C) while those in Experiment V remained in the incubator. Observations on the radicle growing status were made daily for 15 days for the Experiment IV, while for Experiment V observations were made every 2 h in the first 12 h and then once a day for 10 days. With the aid of a lamp and plotting paper, coordinates were recorded for the origin where a radicle penetrates the agar and for the radicle growing point at each observational stage.

Growth angle was defined as the angle formed by a vertical line through the origin (where the radicle penetrates into the agar) and a line through the origin and the growing point of the radicle at each observational time (Figure 1). To make it easier to compare gravitropic measurements, any angle was recorded as a positive angle. The smaller the angle, the greater the gravitropism. Radicle length was defined as the distance from the origin to the growing point at a certain time. Radicle increment was the difference between 2 growing points at each observational period. Growth rate

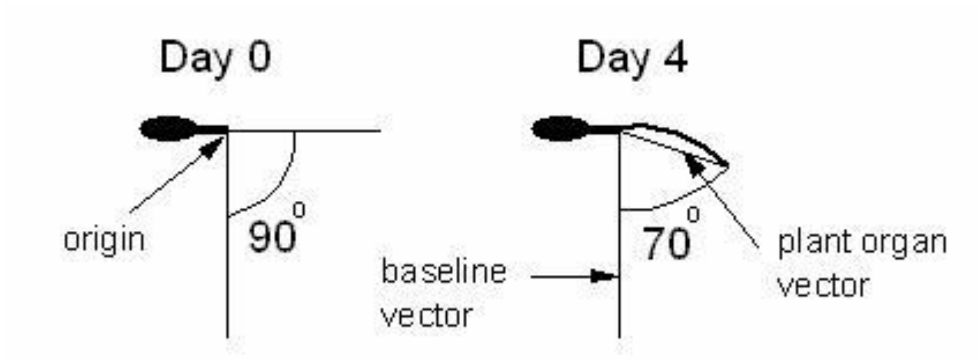

Figure 1. Drawings of the orientation of the seed and radicle at day 0 and day 4. The growth angle is defined as the angle formed by the baseline vector and the plant organ vector. Both vectors pass through the origin (i.e. the position of the radicle tip at the beginning of the experiment).

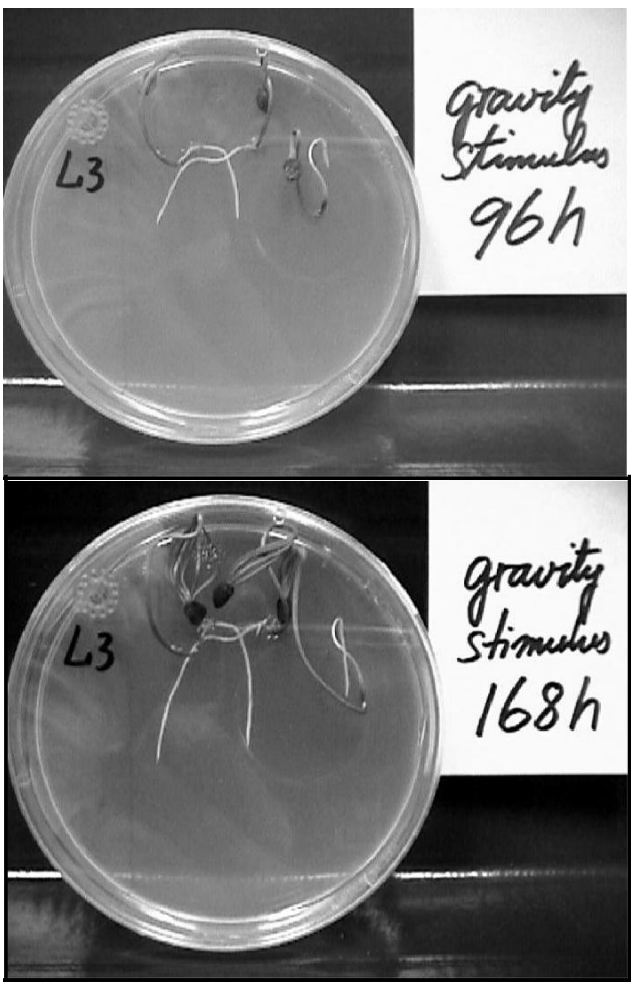

Figure 2. Growth of loblolly pine radicles under sterile growing conditions 96 and 168 h after the dish was rotated 90 degrees (to point the radicle tip horizontally).

was defined as radicle increment per unit of time. Data were analyzed by Statistical Package for the Social Sciences 10.0 (SPSS 10.0), SigmaPlot 5.0, and Excel 2000. Student's t-test was used to test for treatment differences.

RESULTS AND DISCUSSION

Normal gravitropism obtained under sterile conditions

Intact loblolly pine radicles responded to gravity under sterile growing conditions (Figure 2) . They exhibited normal gravitropism 8.3 h (s=6.48 h) after the gravity stimulus was introduced (Figure 3). Average growth angle decreased from 84.5° to 21.2° one week after gravity stimulus introduction. Change in growth angle slowed 72 h after treatment, which suggested that most of the response occurred within the first 3 days. Regression analyses revealed a close relation of growth angle to time. Based upon calculation of SSE (sum of squares for error) and comparison of predicted figures with figures formed by the observed data, cubic and quadratic models were deemed to be appropriate (Table 2). Intact radicles grew normally under sterile growing conditions. Radicle length increased linearly with time, from 0.52 cm to 2.64 cm at the last observational time (Figure 4). Growth rate remained steady at 0.2-0.3 cm per day.

Loblolly pine radicles in $HgCl_2$ treatments exhibited gravitropism 4-11 h ($\bar{x}$ =6.9 h, s=2.42 h) after the petri dish was rotated. There was a continuous decrease in growth angle over time (Figures 5, 6). The angle was 91° (s=9°) at the beginning of Experiment V and 64° (s=16°) after 10 days (Figure 5). Based upon calculation of SSE and comparisons of predicted with observed data, cubic and quadratic models were considered the most appropriate (Table 2). Radicle length increased from 1.5 to 2.3 cm in Experiment V (Figure 7). However, growth rate decreased 24 h after treatment, and 9 days later it was 0.08 cm per day.

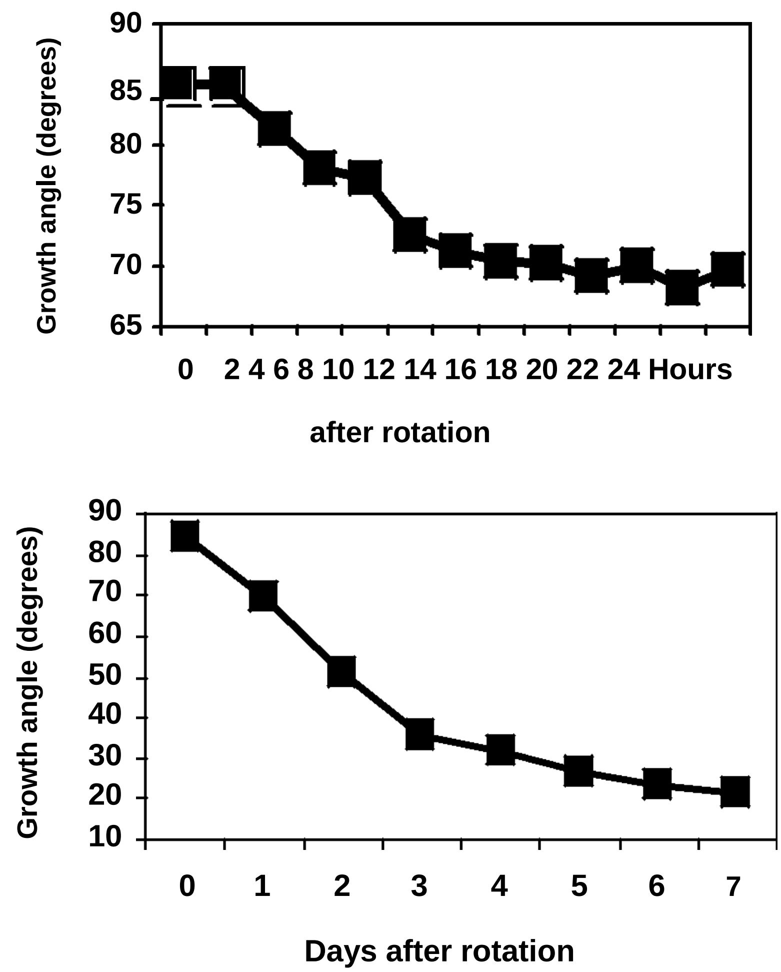

Figure 3. Growth angle of intact loblolly pine radicles during the first week in Experiment I (s=7° at end of study).

Table 2. Models for relating growth angle (Y) of loblolly pine radicles from $HgCl_2$-treated seeds in Experiments IV and V, and from H_2O_2-treated seeds to days after initiation of study (X).

Model	Equation		R^2	*p*-value
$HgCl_2$-treated seeds in Experiment IV	Cubic	$Y=121.066545-0.123114X-0.000895X^2+0.000002X^3$	0.9328	0.0001
	Quadratic	$Y=125.679240-0.307143X+0.000425X^2$	0.9139	0.0001
$HgCl_2$-treated seeds in the Experiment V	Cubic	$Y=91.596503-0.345424X+0.001954X^2-0.000004X^3$	0.9976	0.0001
	Quadratic	$Y=89.516993-0.207752X+0.000450X^2$	0.9686	0.0001
H_2O_2-treated seeds	Cubic	$Y=95.414266-0.002788X-0.000649X^2+0.000001X^3$	0.9952	0.0001
	Quadratic	$Y=96.090979-0.047589X-0.001600X^2$	0.9906	0.0001

Y – growth angle (°).
X – time after gravity stimulus introduction (d).
e = 2.718282.

Radicles from mercuric chloride treated seeds showed a greater expression of gravitropism than those from hydrogen peroxide treated seeds under the similar experimental conditions.

Loblolly pine radicles from H_2O_2-treated seeds started geotropic growth 5 h after dish rotation. Radicle growth angle changed from about 96° to 77° over time. Cubic and quadratic models were the most appropriate models for the data observed (Table 2). Radicle length increased from 2.77 to 3.74 cm over the 10-day period (Figure 6) while growth rate increased from about 0.02 to 0.11 cm per day.

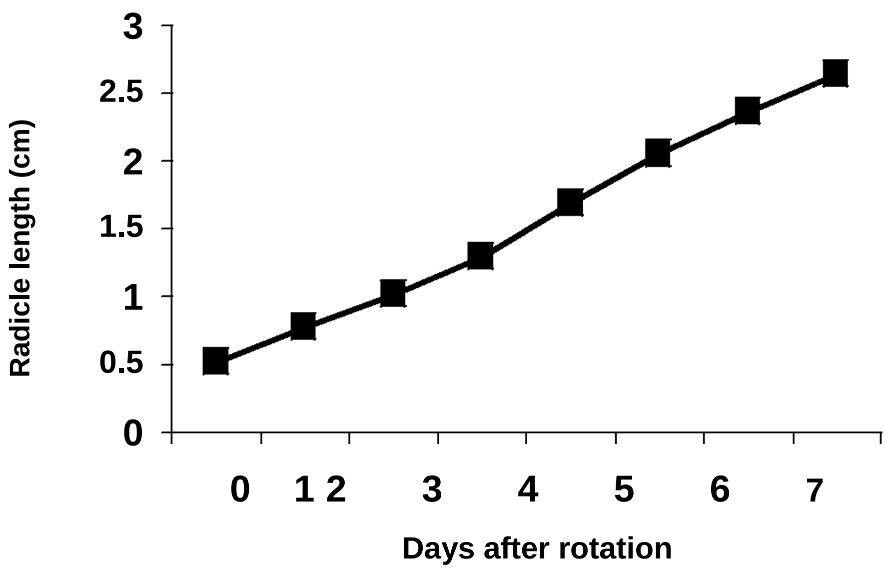

Figure 4. Length of intact loblolly pine radicles during the first week in Experiment I (s=1.9 mm at end of study).

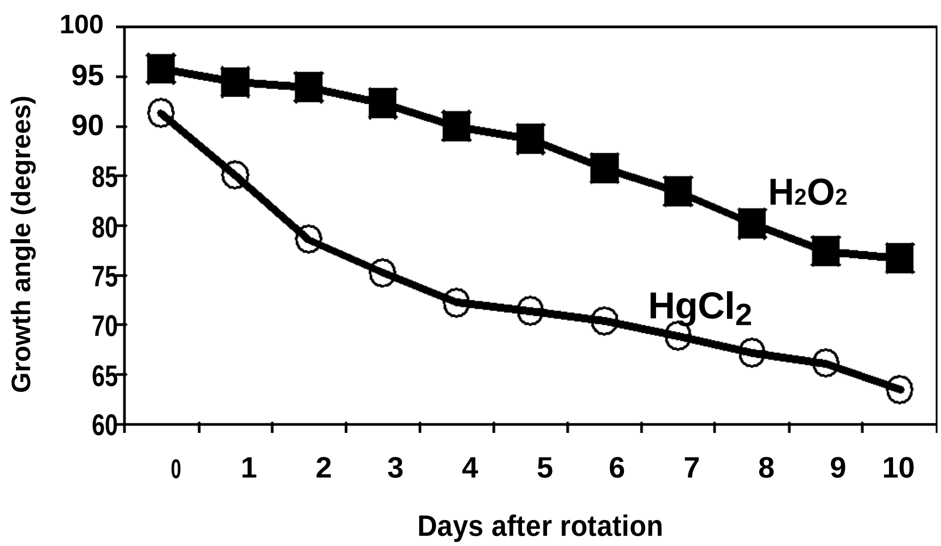

Figure 5. Loblolly pine radicle growth angle over time in different sterilizing treatments in Experiment V (s=5° at end of study). Treatments were significantly different (α=0.05) for days 1 to 10.

Based upon comparison of growth angle and radicle curvature development, radicles from $HgCl_2$-treated seeds showed greater expression of gravitropism than those from H_2O_2-treated seeds under the similar conditions in the experiments (Figure 5) . Eight h after treatment, the difference in growth angle between the two treatments was significant at the 5% level. From 1 to 6 days after treatment, differences were significant at the 1% level.

Mercuric chloride was a more effective sterilant and did not have a negative influence on loblolly pine gravitropism.

Although the applied concentration was lower and treatment time shorter, $HgCl_2$ was more effective than H_2O_2 for sterilizing loblolly pine seeds. Both in Experiments III and V, all 80 seeds were free from fungi, compared to 24% fungal contamination with H_2O_2 in Experiment V. In Experiment IV, only 2 out of 50 seeds treated with $HgCl_2$ were contaminated with fungi. As for bacterium-infested seeds, there was 16%-27% contami- nation in the $HgCl_2$ treatment but contamination in the H_2O_2 treatment was greater than 80%.

While the data indicated that $HgCl_2$ did not have a negative influence on loblolly pine gravitropism, 3 out of

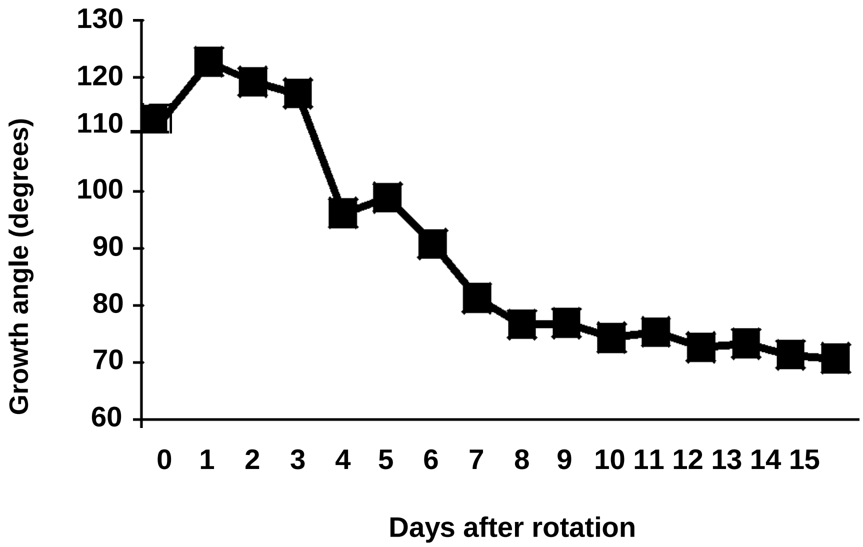

Figure 6. Growth angle of loblolly pine radicles from $HgCl_2$-treated seeds during the first 2 weeks in Experiment IV (s=13° at end of study).

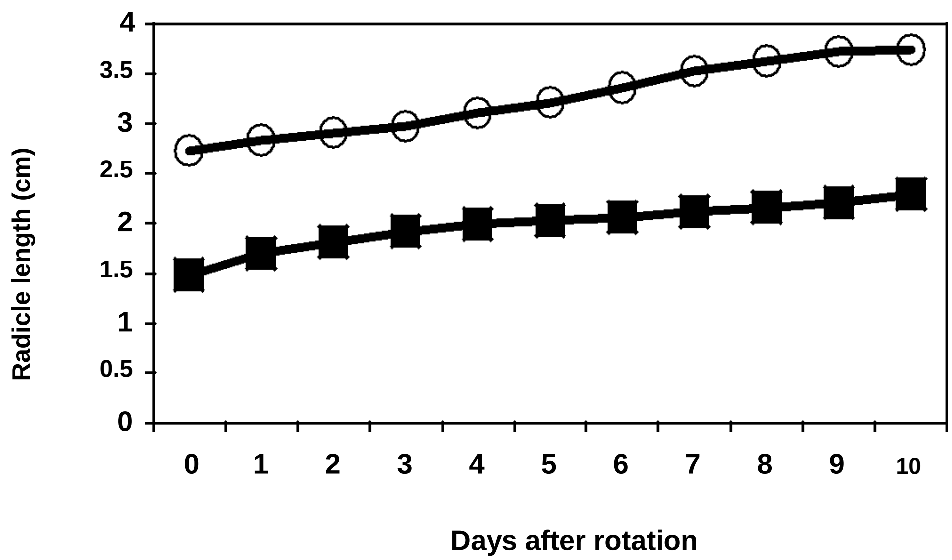

Figure 7. Loblolly pine radicle length over time in different sterilizing treatments (Experiment V) (s=2 mm at end of study). Treatments were significantly different (α=0.05) for days 1 to 10.

the 22 germinated seeds either grew in a twisted manner or were tipless. Heavy metals such as Cu and Zn caused problems with radicle growth in jack pine *(Pinus banksiana* Lamb.)(Govindaraju and Dancik, 1987) . However, it remains unknown if either the cation (Hg^{2+}) or the anion (Cl^-) is harmful to Pinus when used at higher rates.

Future research

It is known that vigorous radicle growth only occurs for a short period of time after germination. Rate of growth declines as the radicle ages. Satisfactory gravitropism observations can be achieved only within a certain time period. It is recommended that a loblolly pine radicle gravitropism tests be completed within 15 days after germination.

In order for seedlings to grow uniformly, only a few germinated seeds (with their radicles approximately 0.5 cm long) should be placed in each petri dish. If seedlings with relatively long radicles are selected, radicle growth will be reduced after transfer to the dish.

Conclusion

These studies confirmed that gravitropism of loblolly pine radicles does occur after seeds have been treated with either $HgCl_2$ or H_2O_2. However, the chemicals selected in the seed treatment can affect the rate and magnitude of gravitropism expression. Researchers who treat pine seeds with chemicals should note that some chemicals can affect the expression of gravitropism.

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