**A Study on the Control of Lead Tree** *Leucaena leucocephala* (Lamark) de Wit] by Herbicides

Tseng-Yu Hwang¹, Ling-Ming Hsu² and Ching-Yuh Wang¹*

¹ Department of Agronomy, National Chung-Hsing University, Taichung 40227, Taiwan ROC
² Division of Plant Toxicity, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung Hsien 41358, Taiwan ROC

**ABSTRACT**

Lead tree *Leucaena leucocephala* (Lamark) de Wit, an exotic plant species, has been widespread in Taiwan since its introduction in the seventeenth century. In this study, dose-response analyses of herbicide susceptibilities of both Hawaii- and Salvardo-type lead trees were carried out. Estimation of control efficacy of eight herbicides with different action mechanisms showed that seed germination of lead tree was more susceptible to glufosinate, a glutamine synthetase inhibitor, and seedling growth was more susceptible to paraquat, a photosystem I inhibitor. Glufosinate, butachlor and fluazifop were also effective in killing seedlings of the examined species. The Hawaii type expressed a slightly higher susceptibility to herbicides than the Salvador type. These experimental results may be taken into consideration in developing chemical control programs for lead trees in the early growth stages.

**Key words:** Seed germination, Seedling injury, Herbicide, Weed control.

**INTRODUCTION**

Lead tree *Leucaena leucocephala* (Lamark) de Wit is native to Central America and the Yucatan Peninsula of Mexico where the Spaniard recognized its fodder value over 400 years ago and carried leucaena feed and seed to the Philippines to feed their stock (Brewbaker 1985). This exotic plant, formerly known as *L. glauca* and introduced into Taiwan in 1643, is a thornless long-lived shrub or tree which may grow to a
height of 7 to 18 m. Its leaves are bipinnate with 6-8 pairs of pinnae bearing 11-23 pairs of leaflets of 8 to 16 mm long. The inflorescence is a cream coloured globular shape which produces a cluster of flat brown pods 13 to 18 mm long containing 15-30 seeds (Shelton and Brewbaker 1994).

There are two types of lead tree found in Taiwan, i.e., the Hawaii and Salvador types. The former is a shrub type with a good adaptation ability to stressed environment and commonly grows to 5 m tall, and the latter is a 20 m giant arbor plant type (Lee 2005). In a lead tree control study in Hengchun Peninsula of southern Taiwan (Chen et al. 2008), more than 95% of the lead trees belong to Hawaii type and only a few plants found near Kuansan village are Salvador type. Recently, due to its ability of vigorous growth, the prevention of this tree from over-propagation islandwide has become an essential issue.

Herbicide hexazinone effectively reduced 75% biomass production in the following season of seedlings of woody plant Pinus taeda L. (Wittwer et al. 1986). In addition, the growth of nine broad-leaf tree seedlings was inhibited with time and the plants were killed 90 days after treatment of herbicide azafenidin (Seifert and Woeste 2002). Hawton et al. (1990) reported that treatment of acifluorfen caused more than 40% dry weight reduction of lead tree seedlings, and it was 21-40% and less than 20% reduction by 2,4-dichlorophenoxyacetic acid (2,4-D) and bentazon and sethoxydim, respectively, and azafenidin showed a satisfied result in lead tree control. In Taiwan, injection of glyphosate into adult lead tree plants gave a better control effect, based on the whole plant wilting or partial wilting 30 days after treatment; however, this effect decreased with time (Wang and Hung 2005). The proper timing for controlling the lead tree was at the stages of seed germination and seedling growth. Wang and Hung (2005) also showed that the effect of glyphosate alone on lead tree control is better than that of a mixture of glyphosate and triclopyr. In addition, herbicides 2,4-D, acifluorfen, bentazon, fluazifop and sethoxydim have been used to control lead tree in Australia (Hawton et al. 1990) and triclopyr has been applied in USA (Kline and Duquesnel 1996). However, a comparison of lead tree control effects among different herbicides is still lacking.

The objective of this study was to estimate the control efficacy toward lead tree using eight herbicides with different action mechanisms, based on the dose-response analyses, at seed germination and seedling stages. The experimental results may be useful for herbicide selection in lead tree control in the early growth stage.

**MATERIALS AND METHODS**

**Response of Seed Germination to Herbicides**

Seeds of both Hawaii- and Salvador-type lead tree were used as materials in a dose-response analysis (Seefeldt et al. 1995). Three independent experiments were conducted, and each experiment had three replicates with 20 seeds per replicate. Eight herbicides with different action mechanisms were adopted, including 44.1% bentazon (a photosystem II inhibitor, BASF Co., Ludwigshafen, Germany), 32% butachlor (a pre-emergence selective systemic herbicide, Yih-Fong Chemicals, Taichung, Taiwan), 40% 2,4-D ammonium salt (an auxin-type herbicide, Jia-Bin, Tainan, Taiwan), 17.5% fluazifop (an acetyl coenzyme A carboxylase inhibitor, Taiwan Farmers’ Association, Yunlin, Taiwan), 13.5% glufosinate (a glutamine synthetase inhibitor, Bayer in Taiwan, Taipei, Taiwan), 41% glyphosate isopropyl ammonium salt (an aromatic amino acid synthesis inhibitor, Yih-Fong Chemicals, Taichung, Taiwan), 40% 2,4-D ammonium salt (an auxin-type herbicide, Jia-Bin, Tainan, Taiwan), 17.5% fluazifop (an acetyl coenzyme A carboxylase inhibitor, Taiwan Farmers’ Association, Yunlin, Taiwan), 13.5% glufosinate (a glutamine synthetase inhibitor, Bayer in Taiwan, Taipei, Taiwan), 41% glyphosate isopropyl ammonium salt (an aromatic amino acid synthesis inhibitor, Yih-Fong Chemicals, Taichung, Taiwan), 24% paraquat (a photosystem I inhibitor, Syngenta, Taipei, Taiwan), and 10% pyrazosulfuron-ethyl (a branched-chain amino acid synthesis inhibitor, Nissan Chemical Industries, Taipei, Taiwan).

All herbicides were diluted to 1/10, 1/50, 1/100, 1/1,000, 1/5,000, 1/10,000 and 1/50,000 folds from the commercial products, and a 5 mL aliquot of the herbicide solutions was applied on the filter paper (filter paper 5C, Toyo Roshi Kaisha, Ltd., Japan) placed at the bottom of a petri dish (dia. 9 cm). All dishes were sealed with parafilm before incubating at 35°C in dark for 7 days. The seed germination rate of lead tree was assessed and ED_{50} values were calculated based on the log-logistic model of dose-response analysis (Seefeldt et al. 1995).
Response of Seedling Growth to Herbicides

Seedlings of 10 cm in height of both Hawaii- and Salvador-type lead tree were used as materials. Different concentrations of each herbicide as described previously in seed germination experiment were foliar sprayed on lead tree seedling, and the injury index of whole plant was measured in 1, 2, 3, 4, 5, 7 and 14 days after treatment (DAT). Injury index ranged from 0 to 5, with 0 indicating normal plant without herbicidal injury and 5 indicating dead plant. The ED_{50} values based on the injury index were calculated by fitting the log-logistic model of dose-response analysis (Seefeldt et al. 1995).

All experiments were conducted 3 times with treatments replicated at least 3 times in each study. Data are presented as the mean and standard error.

RESULTS AND DISCUSSION
Response of Seed Germination to Herbicides

Dose-response analysis of seed germination to different herbicides 7 DAT showed that the ED_{50} of seed germination in Salvador type to glufosinate at 3.97 mM was the minimum, when compared with the remaining seven herbicides (Table 1), followed by paraquat at 5.14 mM, fluazifop at 9.97 mM, butachlor at 10.22 mM, 2,4-D at 10.45 mM, bentazon at 11.33 mM, pyrazosulfuron-ethyl at 13.65 mM and glyphosate at 30.31 mM. That is, seed germination of lead tree was most sensitive to glufosinate and most tolerant to glyphosate. Although both herbicides are amino acid synthesis inhibitors, the target protein of the former is glutamine synthetase which is the key enzyme of all amino acid synthesis in plants; whereas, the latter is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is only responsible for the biosynthesis of three aromatic amino acids.

Besides, it was found that seed germination was moderately sensitive to pyrazosulfuron-ethyl, an acetolactate synthase (ALS) inhibitor, which controls the biosynthesis of three branched-chain amino acids in plants. Similar trend of seed germination response to three herbicides existed in Hawaii type. Results imply that biosynthesis of some branched-chain amino acids, rather than aromatic amino acids, was a more crucial process involved in seed germination of lead tree. Despite an earlier study reported that seed germination of lead tree in seedbed was decreased to less than 10% by glyphosate 28 days after treatment (Thomson et al. 1983), herbicides with better control percentages than glyphosate are found in this study.

Response of Seedling Growth to Herbicides

Dose-response analysis based on the plant injury of 3-leaf seedlings to different herbicides 14 DAT showed that the ED_{50} of Salvador type seedling growth to paraquat at 1.02 mM was the minimum, when compared with the other seven herbicides (Table 2), followed by glufosinate at 2.92 mM, glyphosate at 5.10 mM, fluazifop at 5.80 mM, butachlor at 8.61 mM, 2,4-D at 11.01 mM, pyrazosulfuron-ethyl at 13.21 mM, and bentazon at 39.03 mM. In other words, seedling growth is more sensitive to paraquat, an inhibitor of photosystem I (PS I), and more tolerant to bentazon, an inhibitor of PS II. Although both paraquat and bentazon are photoreaction inhibitors in photosynthesis, the target protein of the former is NADPH-dependent ferredoxin reductase, whereas the primary action of the latter is to compete for the binding site (Q_{b}) of the D1 protein with plastoquinone and results in the inhibition of photosynthetic electron transport and the promotion of chlorophyll degradation (Burton and Maness 1992, Han and Wang 2002, McFadden et al. 1990). Besides, paraquat has been reported to affect cell function and cause mitochondrial damage (Costantini et al. 1995). This study indicates that the inhibitor of PSI electron transport seems more toxic to lead tree seedlings than PSII inhibitor. Similar results appeared in Hawaii-type lead tree. Thus, paraquat is highly recommended to control lead tree seedlings.

Regarding the amino acid synthesis of lead tree seedlings under treatments of three amino acid synthesis inhibitors, i.e., glutamine synthetase (GS), acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors, GS and EPSPS were seemly more crucial for seedling growth than ALS, and
Table 1. Dose-response of lead tree to eight herbicides with different action mechanisms, based on seed germination rate 7 days after treatment.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Action mechanism</th>
<th>ED\textsubscript{50}</th>
<th>Salvador</th>
<th>Hawaii</th>
<th>LSD\textsubscript{0.05}x</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>Auxin-type</td>
<td>10.45</td>
<td>9.14</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Bentazon</td>
<td>PS II\textsuperscript{y} inhibitor</td>
<td>11.33</td>
<td>10.09</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Butachlor</td>
<td>Unknown</td>
<td>10.22</td>
<td>8.06</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Fluazifop</td>
<td>ACCase inhibitor</td>
<td>9.97</td>
<td>8.11</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>GS inhibitor</td>
<td>3.97</td>
<td>2.51</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>EPSPS inhibitor</td>
<td>30.31</td>
<td>25.45</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Paraquat</td>
<td>PS I inhibitor</td>
<td>5.14</td>
<td>4.58</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Pyrazosulfuron-ethyl</td>
<td>ALS inhibitor</td>
<td>13.65</td>
<td>11.05</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
<td></td>
<td>13.86</td>
<td>11.72</td>
<td></td>
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</tbody>
</table>

\textsuperscript{x} Fisher’s protected least significant difference (LSD) at 5% level.

\textsuperscript{y} PS II: photosystem II; ACCase: acetyl-CoA carboxylase; GS: glutamine synthetase; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; PS I: photosystem I; ALS: acetolactate synthase.

Table 2. Dose-response of 3-leaf seedlings of lead tree to eight herbicides with different action mechanisms, based on the injury index of whole plants 14 days after treatment.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Action mechanism</th>
<th>ED\textsubscript{50}</th>
<th>Salvador</th>
<th>Hawaii</th>
<th>LSD\textsubscript{0.05}x</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>Auxin-type</td>
<td>11.01</td>
<td>1.89</td>
<td>7.57</td>
<td>_</td>
</tr>
<tr>
<td>Bentazon</td>
<td>PS II\textsuperscript{y} inhibitor</td>
<td>39.03</td>
<td>29.87</td>
<td>ND</td>
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<tr>
<td>Butachlor</td>
<td>Unknown</td>
<td>8.61</td>
<td>6.16</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Fluazifop</td>
<td>ACCase inhibitor</td>
<td>5.80</td>
<td>3.96</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>GS inhibitor</td>
<td>2.92</td>
<td>1.80</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>EPSPS inhibitor</td>
<td>5.10</td>
<td>3.73</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Paraquat</td>
<td>PS I inhibitor</td>
<td>1.02</td>
<td>1.03</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>Pyrazosulfuron-ethyl</td>
<td>ALS inhibitor</td>
<td>13.21</td>
<td>13.37</td>
<td>ND</td>
<td>_</td>
</tr>
<tr>
<td>LSD\textsubscript{0.05}</td>
<td></td>
<td>11.49</td>
<td>11.04</td>
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</tr>
</tbody>
</table>

\textsuperscript{x} Fisher’s protected least significant difference (LSD) at 5% level.

\textsuperscript{y} PS II: photosystem II; ACCase: acetyl-CoA carboxylase; GS: glutamine synthetase; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; PS I: photosystem I; ALS: acetolactate synthase.

these changes in amino acid synthesis of lead tree seedlings were different from those in germinating seeds. Similar results of amino acid synthesis response to herbicides were also observed in Hawaii-type lead tree. Therefore, it is suggested that biosynthesis of aromatic amino acids, rather than branched-chain amino acids, were the critical process during seedling growth of lead tree. Based on the consistent results of glufosinate effect on seed germination and seedling injury, this herbicide is recommended to control lead tree in the early growth stages.

In order to assess the damage in the development of lead tree seedlings after chemical herbicide application, changes in ED\textsubscript{50} values of each herbicide within 14 DAT were compared. No significant difference of plant injury in response to each herbicide was observed between Salvador and Hawaii types (Fig. 1). The ED\textsubscript{50} of paraquat remained the lowest among all herbicides tested.
Fig. 1. Changes in ED$_{50}$ values of seedlings for Hawaii (●) and Salvador (○) types of lead tree [Leucaena leucocephala (Lamark) de Wit] to eight herbicides with different action mechanisms.
from 1 to 14 DAT, indicating that this herbicide is the best choice for the control of lead tree seedlings. Bentazon, another photosynthesis inhibitor, showed a similar rapidity, but the higher ED50 value implied a lower efficacy. Among the three inhibitors of amino acid synthesis, glufosinate displayed the highest efficacy and rapidity, relative to glyphosate and pyrazosulfuron-ethyl (Fig. 1). The 2,4-D, an auxin-type herbicide, had a slow effect like glyphosate and pyrazosulfuron-ethyl. Both fluazifop and butachlor expressed rapid effect in controlling lead tree seedlings. Although 2,4-D was reported to give a better lead tree control than bentazon or sethoxydim (Hawton et al. 1990), this study found the herbicidal effect of 2,4-D was slower than that of bentazon based on the results of dose-response analysis.

In summary, seed germination of lead tree was more susceptible to glufosinate, a GS inhibitor, and seedling growth was more susceptible to paraquat, a PS I inhibitor. Glufosinate, butachlor and fluazifop also exerted fast and efficient effects for controlling seedling growth of lead trees. The experimental results offer useful information for the selection of herbicides to be applied in the early growth stages of lead tree.

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REFERENCES


