A new method for screening herbicides for use in pine nurseries

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A new method for screening herbicides for use in pine nurseries

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A rapid bioassay developed for testing photosynthesis-inhibiting herbicides was adapted for use with pine cotyledon tissue. Several herbicides having different modes of action were tested using loblolly pine (*Pinus taeda* L.) cotyledons. In addition to being a promising bioassay method for determining pine tolerance of photosynthesis inhibitors, differences in pine species' tolerance to hexazinone could be detected. This method could be useful as a preliminary test for screening herbicides for use in pine nurseries.

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Un test biologique rapide, mis au point pour évaluer l'inhibition de la photosynthèse par les herbicides, a été adapté pour utilisation avec le tissu des cotylédons de pin. Plusieurs herbicides ayant différents modes d'action ont été expérimentés sur les cotylédons du pin à feuilles tordues (*Pinus taeda* L.). En plus de constituer un test biologique prometteur dans la détermination de la toléraance d'un pin en regard de l'inhibition de la photosynthèse, la méthode permet de détecter chez les pins différents niveaux de tolérance vis-à-vis l'hexazinone. Cette méthode pourrait s'avérer utile comme test préliminaire pour le choix des herbicides à utiliser en pépinière.

[Traduit par le journal]

Introduction

Screening of herbicides for use in forest nurseries is often a time-consuming process requiring a large expenditure of manpower, money, and time. Often the number of compounds which can be screened per year is limited because of inadequate greenhouse space. It would be desirable to have a simple test which would indicate if the photosynthetic or respiratory processes of a seedling were adversely affected by a given compound.

Truelove *et al.* (12) described a simple bioassay for determining photosynthesis inhibition using discs cut from the cotyledons of pumpkin (*Cucurbita pepo* L.). The method is based on observation that discs float in the light and sink in the dark in check solutions. However, in the presence of herbicides that decrease oxygen evolution, discs sink in both light and dark. The buoyancy of the discs in controls in light is apparently related to the lower solubility of oxygen compared with that of carbondioxide in water. If photosynthesis is occurring, the concentration of oxygen in the intercellular spaces of discs is high and they float. The loss of buoyancy in the absence of photosynthesis is believed to be due to a depletion of this oxygen through respiration followed by the gradual filling of the spaces with the surrounding liquid.

This method has also been used as a screening test for potential respiration inhibitors. It has been shown that when respiration was inhibited, the discs maintained buoyancy even in the dark (2).

This work evaluates the adaptation of this technique for use with pine cotyledons and examines cotyledon responses to several herbicides having different modes of action.

Materials and methods

In the first study, loblolly pine seeds (*Pinus taeda* L.) were planted in Jiffy® mix in metal trays, watered, and allowed to germinate in a 28°C greenhouse. After the cotyledons were fully expanded and primary needles were developing (approximately 35 days after planting), 5-mm segments of the cotyledons were cut and transferred to distilled water. Fifty of the cotyledon segments were selected at random and transferred to 250-mL beakers containing 100 mL of a 1×10^{-3} *M* solution of the herbicide to be evaluated. To ensure solubility, herbicides were first dissolved in a small amount of acetone (final concentration of acetone in the medium was less than 1%, v/v). A similar volume of acetone was also added to the controls. The beakers containing treatments requiring darkness were wrapped in aluminum foil. All treatments including the controls were replicated three times. Beakers requiring light were covered with watch glasses and illuminated at an intensity of 30 klx at approximately 28°C. Prior to recording the number of sunken segments, all floating segments were immersed in the solution to ensure that surface tension was not maintaining the segments afloat.

In the second study, a comparison was made of the response of different pine species to hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione). Seeds of loblolly pine, longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*Pinus echinata* Mill.), and eastern white pine (*Pinus strobus* L.) were grown in germination packets in a growth chamber having 12-h days at 23°C with 50% relative humidity and 500 lx of light, and 12-h nights at 17°C and 70% relative humidity. Soon after enough seedlings had germinated and cotyledons were fully expanded (approximately 21 days after planting), cotyledon segments were cut and transferred to water and to solutions containing 1×10^{-4} *M* hexazinone. Conditions were similar to the first experiment except that no acetone was used to prepare the herbicide solutions because the herbicide is water-soluble.

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The methods presented here differ from those of Truelove *et al.* (12) in two ways. First, the beakers were not shaken, and second, no antibiotics or buffers were added to the test media.

An analysis of variance (*F*-test) was performed for each experiment using an arcsine transformation of the percentage of segments from each treatment which had sunk. Duncan's multiple range test was then used to compare individual means for significant differences. In the tables, the numbers represent the percentage of discs sunk for various time intervals after each treatment.

Results and discussion

First experiment

Herbicides evaluated in the first experiment were selected because the tolerance of loblolly pine seedlings to these herbicides is known (3, 4, 7, 9, 10, 11). The tolerance to these herbicides appears to correspond closely to the rates of sinking cotyledon segments in the light (Fig. 1).

Triazine herbicides are inhibitors of photosynthesis (1). The three triazine herbicides, hexazinone, atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine), and prometryn (2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine) all have been known to injure loblolly pine seedlings (3, 4, 9). A greenhouse study has indicated that atrazine is more toxic to loblolly than prometryn (3). Atrazine at 3.4 kg/ha reduced survival of loblolly by 83% whereas prometryn at 3.4 kg/ha did not decrease survival. Although prometryn has been used at a number of nurseries in the South, an application of 2.2 kg/ha has injured seedlings (4). Hexazinone is more toxic to loblolly than atrazine or prometryn because only 0.6 kg/ha can cause 99% mortality (9).

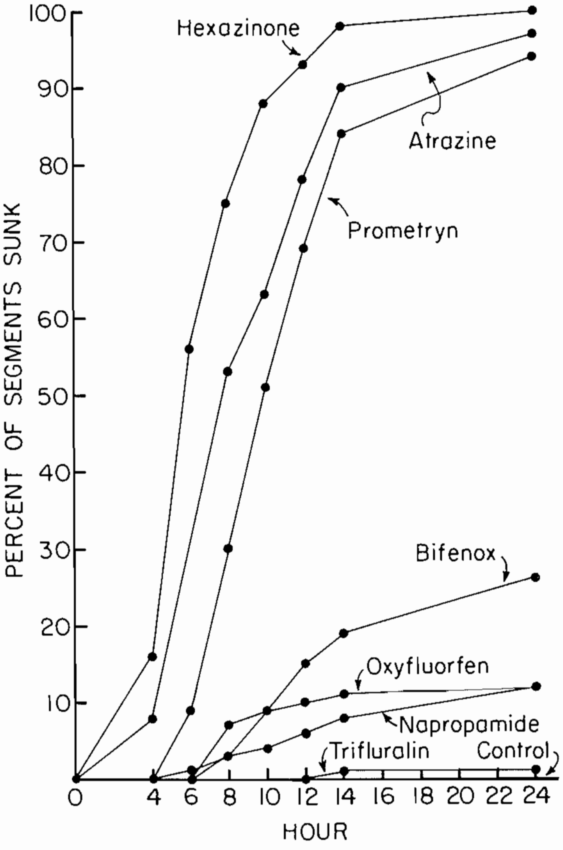

FIG. 1. The effect of seven herbicides at 1×10^{-3} *M* concentration on sinking of loblolly pine cotyledon segments in the light.

Table 1 shows that in the light, all three triazines induced sinking of the cotyledons. The time required for the triazines to cause a significant difference from the light control was directly related to the tolerance of loblolly to these herbicides. Hexazinone and atrazine are the most toxic and increased sinking in 4 h. Prometryn, the least toxic of the three triazines, did not increase sinking before 8 h. Hexazinone, more toxic than either atrazine or prometryn, caused more sinking than the other triazines at all measurement periods.

Although several diphenyl ether herbicides have been shown to be moderate inhibitors of photosynthesis in isolated chloroplasts (8), bifenox (methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate) and oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluormethyl)benzene) are apparently safe on loblolly pine (10, 11). Germination was not decreased by bifenox at 6.7 kg/ha or by oxyfluorfen at 1.1 kg/ha. Survival of 4-week-old seedlings was not decreased with bifenox at 4.5 kg/ha or oxyfluorfen at 1.1 kg/ha. Although oxyfluorfen caused some sinking of cotyledon tissue in the light, the difference from the light control was not statistically significant (Table 1). Except for the 24-h measurement, bifenox was also not different from the light control. This suggests that diphenyl ether herbicides affect photosynthesis of loblolly pine to a much lesser extent than the triazine herbicides.

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is a dinitroanaline herbicide which is a mitotic poison and inhibits root growth, but it does not affect photosynthesis (1). When applied after sowing, trifluralin at 1.1 kg/ha did not affect the survival of lobolly pine (4,7). As expected, trifluralin did not significantly affect the rate of sinking cotyledons in the light.

Napropamide (2-(α-naphthoxy)-*N*,*N*-diethylpropionamide) is a substituted amide herbicide which inhibits root growth (13). This herbicide is being used on loblolly at a number of nurseries in the South. However, at one nursery having soil with low organic matter, survival of loblolly pine was

TABLE 1. Effect of seven herbicides on the sinking of loblolly pine cotyledon tissue

	% segments sunk at various times after treatment*						
Treatment†	4 h	6 h	8 h	10 h	12 h	14 h	24 h
Light							
Control	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Bifenox	0 *a*	0 *a*	3 *a*	9 *a*	15 *a*	19 *a*	26 *b*
Oxyfluorfen	1 *a*	5 *a*	7 *a*	9 *a*	10 *a*	11 *a*	12 *ab*
Atrazine	8 *b*	30 *b*	53 *c*	63 *b*	78 *b*	90 *b*	97 *c*
Hexazinone	16 *c*	56 *c*	75 *d*	88 *c*	93 *c*	98 *c*	100 *d*
Prometryn	0 *a*	9 *a*	30 *b*	51 *b*	69 *b*	84 *b*	94 *c*
Napropamide	0 *a*	1 *a*	3 *a*	4 *a*	6 *a*	8 *a*	12 *ab*
Trifluralin	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	1 *a*	1 *a*
Dark							
Control	4 *h*	12 *hi*	19 *h*	33 *hi*	39 *hi*	53 *hi*	59 *h*
Bifenox	0 *h*	7 *h*	15 *h*	25 *h*	35 *h*	52 *h*	66 *h*
Oxyfluorfen	20 *i*	44 *j*	66 *j*	76 *j*	85 *k*	91 *jk*	98 *k*
Atrazine	7 *h*	30 *j*	50 *hij*	55 *i*	66 *j*	79 *ij*	88 *ij*
Hexazinone	6 *h*	35 *j*	58 *ij*	75 *j*	86 *k*	93 *kl*	97 *ik*
Prometryn	1 *h*	11 *hi*	29 *hi*	43 *hi*	60 *ij*	73 *i*	87 *i*
Napropamide	10 *hi*	33 *ij*	69 *j*	87 *j*	94 *k*	99 *l*	99 *k*
Trifluralin	7 *h*	39 *j*	59 *ij*	77 *j*	87 *k*	94 *kl*	100 *k*

*Values within each column and light treatment followed by the same letter are not significantly different at the 5% level as judged by Duncan's multiple range test.

†All chemicals were used at a final concentration of 1×10^{-3} *M*.

TABLE 2. Effect of 1×10^{-4} *M* hexazinone in the dark and light on the sinking of cotyledon tissue of four pine species

	% segments sunk at various times after treatment*						
Treatment	4 h	6 h	8 h	10 h	12 h	14 h	24 h
Light, no hexazinone							
Longleaf	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Loblolly	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Shortleaf	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Eastern white	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Light, hexazinone							
Longleaf	1 *g*	5 *h*	12 *l*	26 *l*	41 *h*	69 *l*	97 *g*
Loblolly	1 *g*	5 *h*	23 *h*	44 *h*	52 *h*	77 *hl*	98 *g*
Shortleaf	1 *g*	14 *g*	54 *g*	66 *g*	74 *g*	82 *gh*	99 *g*
Eastern white	3 *g*	14 *g*	59 *g*	71 *g*	80 *g*	87 *g*	99 *g*
Dark, no hexazinone							
Longleaf	0 *m*	2 *m*	3 *m*	5 *n*	7 *n*	18 *n*	87 *o*
Loblolly	3 *m*	6 *m*	10 *m*	14 *mn*	21 *mn*	35 *n*	93 *mn*
Shortleaf	0 *m*	1 *m*	4 *m*	9 *mn*	16 *mn*	36 *n*	91 *no*
Eastern white	4 *m*	6 *m*	16 *m*	28 *m*	38 *m*	74 *m*	96 *m*
Dark, hexazinone							
Longleaf	1 *t*	2 *t*	7 *u*	12 *u*	22 *u*	41 *u*	92 *u*
Loblolly	0 *t*	4 *t*	21 *tu*	29 *tu*	42 *tu*	67 *t*	95 *t*
Shortleaf	4 *t*	10 *t*	28 *t*	37 *t*	48 *st*	65 *t*	90 *u*
Eastern white	11 *s*	24 *s*	46 *s*	58 *s*	66 *s*	84 *s*	100 *s*

*Values within each column and treatment followed by the same letter are not significantly different at the 5% level as judged by Duncan's multiple range test.

reduced when treated with a preemergence application of 3.4 kg/ha (7). Napropamide caused some sinking of cotyledon segments in the light, but the difference from the light control was not significant.

These results indicate that this method may be suitable for screening photosynthesis inhibitors for use in pine seedbeds. Photosynthesis inhibitors which cause a rapid sinking of cotyledon segments in the light are likely to be too toxic for newly emerged seedlings. However, older seedlings having mainly true needles (such as transplants) may be more tolerant of these same chemicals. Both young and old seedlings should be tolerant of photosynthesis inhibitors which do not significantly increase sinking. When using this technique to screen herbicides for use in pine seedbeds, standards such as prometryn and bifenox should be included as references.

Carter and Camper (2) reported that, when in the presence of a respiratory inhibiting chemical, buoyancy of pumpkin cotyledon discs is maintained in the dark. A preliminary test with a 1 × 10^{-3} M solution of potassium azide demonstrated this to also be true for loblolly pine because no cotyledon segments sank after 24 h in the dark. However, of the seven herbicides tested, none decreased dark respiration because no dark treatment maintained cotyledon buoyancy (Table 1). In fact, by the 14-h measurement, all herbicides except bifenox were causing the cotyledon segments to sink faster than the dark control. This suggests that these herbicides may be affecting some metabolic process which increases dark respiration.

It has been reported that diphenylethers like bifenox and oxyfluorfen require light for herbicidal activity (5). In our experiment, bifenox in the dark was no different than the dark control. However, oxyfluorfen more than tripled the rate of sinking during the first 8 h. Apparently, oxyfluorfen does increase respiration in the absence of light. Evidently, the increased respiration does not result in phytotoxic injury of loblolly. Because loblolly is tolerant of oxyfluorfen, screening of herbicides by comparing differences in increased rate of sinking in the dark is not reliable.

Second experiment

Observations from nursery experiments have indicated that some pine species are more sensitive to herbicide injury than others. Apparently shortleaf is more sensitive to perfluidone, butralin, and trifluralin than loblolly.[2] In addition, eastern white pine is injured by prometryn at 1.1 kg/ha while loblolly is unaffected.[3] Since the sinking disc technique has been utilized to detect differences in herbicide tolerance among potato (*Solanum tuberosum* L.) varieties (6), this experiment was conducted to determine if differences among pine species could be detected. The results in Table 2 indicate that longleaf is more tolerant of hexazinone than loblolly and that shortleaf and eastern white pine are the least tolerant.

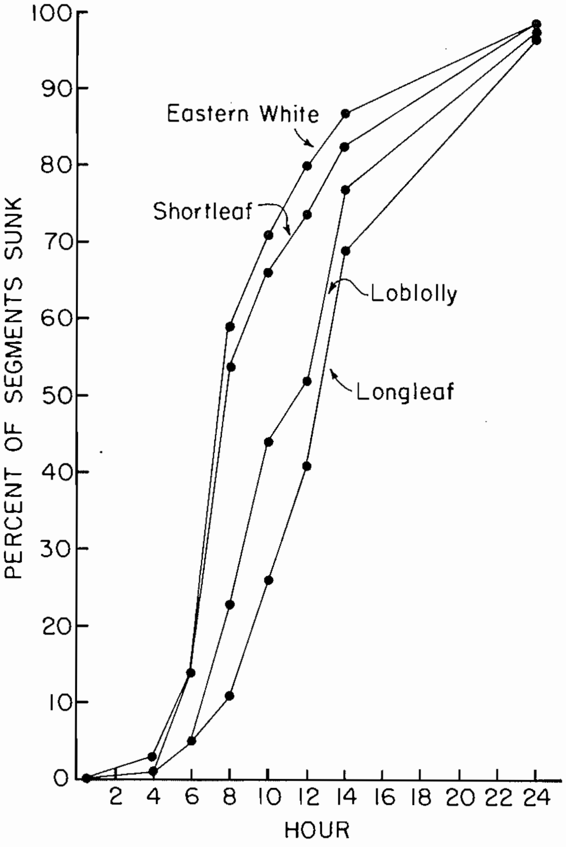

FIG. 2. The effect of hexazinone at 1 × 10^{-4} M concentration on sinking of cotyledon segments from four pine species in the light.

When comparing the light treatments (Fig. 2), differences in species tolerance became more noticeable with time. At the 8- and 10-h measurements, longleaf was less affected by hexazinone than loblolly which in turn was less affected than either shortleaf or eastern white pine.

Results from the dark hexazinone treatments indicate that respiration of all four species is possibly increased. The sequence of species response was

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[2] Gjerstad, D. H., and D. B. South. 1977. Auburn University Forest Chemicals Cooperative annual report. Dep. For., Auburn Univ., Auburn, AL.

[3] Gjerstad, D. H., and D. B. South. 1976. Cooperative Forest Nursery Weed Control annual report. Dep. For., Auburn Univ., Auburn AL.

similar to the response in the light. Eastern white pine was the first to be affected after 4 h. At the 8- and 10-h measurements, longleaf was less affected than shortleaf which in turn was less affected than eastern white pine. Loblolly was intermediate between longleaf and shortleaf. Although the results from this experiment indicate differing tolerances among pine species to herbicides, greenhouse or field tests with seedlings will have to be conducted in order to verify these results.

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