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Symptomatic effects of dichlobenil on three species of needled evergreens.
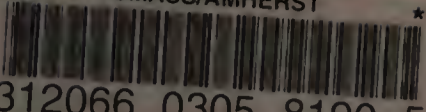
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SYMPTOMATIC EFFECTS OF DICHLOBENIL ON

THREE SPECIES OF NEEDLED EVERGREENS

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Thesis submitted in partial fulfillment

of the requirements of the degree of

Master of Science

Department of Plant and Soil Sciences

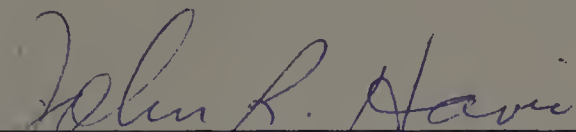University of Massachusetts

August   1970

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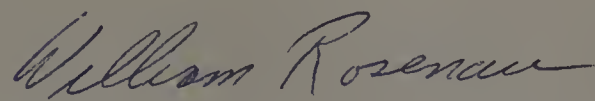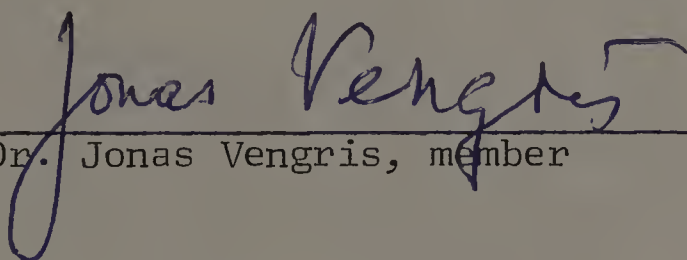# THREE SPECIES OF NEEDLED EVERGREENS

A Thesis

by

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Approved as to style and content by:

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INTRODUCTION

The use of herbicides in crop production has become almost a necessity with today's agricultural mechanization and high labor costs. Thus it becomes important for agricultural and chemical research stations to study the usefulness of herbicides against weed pests in particular crops and to try to better understand their mode of action in plants.

The herbicide 2,6-dichlorobenzonitrile (dichlobenil) was chosen to be studied because of its apparent effectiveness against certain important weeds, especially around woody plants. In addition, the toxicity symptoms, sites of penetration, and mode of activity of dichlobenil in plants are not fully understood. Occasional reports of injury to certain ornamental plants, especially some needled evergreens, emphasize the need to better understand the nature of injury to the plant in relation to application rate and placement of the herbicide.

The objective of this study was to describe the morphological and anatomical effects of dichlobenil on stems and roots of certain needled evergreens in response to varying rates and sites of contact of the herbicide.

LITERATURE REVIEW

The chemical 2,6-dichlorobenzonitrile has been assigned the common name dichlobenil by the British Standards Institution, the Weed Science Society of America, and the Pesticide Regulation Division of the United States Department of Agriculture. Dichlobenil has been marketed as an herbicide under the trade name Casoron by Philips Roxane in Europe and by the Thompson-Hayward Chemical Company in North America.

2,6-Dichlorobenzonitrile was first recognized as an herbicide by Koopman and Daam in 1960 at the N.V. Philips Duphar Research Laboratories (38). They found that it inhibited potato sprout development and therefore began testing the chemical for herbicidal activity.

Physical Properties

2,6-Dichlorobenzonitrile is a white crystalline solid with a melting point of 142°C. It has a vapor pressure of 5×10^{-4} mm Hg at 20°C and has a solubility in water of approximately 18 parts per million. The evaporation half-life of 100 mg of crystalline material at 40°C is 90 hours (6).

The absorption coefficients of dichlobenil on various substances are listed below.*

Absorbant	$k = \dfrac{\text{weight of dichlobenil in absorbant}}{\text{weight of dichlobenil in liquid phase}}$
Cellulose	1.0
Lignin	400 - 1000

Chloroplast (Phaseolus vulgaris) 230

Stems (Phaseolus vulgaris)   50

Roots (Phaseolus vulgaris)   80

Potting soil   180
    (22% organic matter)

Sand   0.4

*Adapted from Massini (42)

The formula of dichlobenil is presented below.
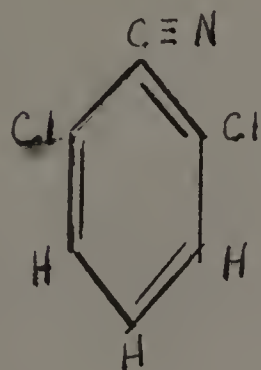
Weed Control Properties of Dichlobenil

Dichlobenil has been shown to control many noxious weeds. A partial list of weeds that dichlobenil controls follows:

Weeds controlled	Reference
Annual bluegrass (Poa annua)	7,40
Aster (Aster spp.)	18
Common chickweed (Stellaria media)	23
Crabgrass (Digitaria)	40,46
Dandelion (Taraxacum spp.)	7,40
Dodder (Cuscuta spp.)	17

Horsetail (Equistum spp.)   6

Loosestrife (Lythrum spp.)   6

Orchardgrass (Dactylis glomerata)   39

Plantain (Plantago spp.)   40,41

Poison Ivy (Rhus radicans)   20

Purple nutsedge (Cyperus esculentus)   5

Quackgrass (Agropyron repens)   1,39

Ragweed (Ambrosia spp.)   18

Rice cutgrass (Leersia oryzoides)   6

Royal fern (Osmunda regalis)   18

Sensitive fern (Onoclea sensibilis)   18

Sorrel (Rumex acetosa)   18

Spanish needles (Bidens bipinnata)   6

Sweet vernalgrass (Anothoxanthum odoratum)   41

Yellow nutgrass (Cyperus esculentus)   18

Most of these weeds can be controlled by several herbi-
cides. Quackgrass, however, is a very difficult weed to
kill and one that can cause much damage both to agronomic
crops (37) and to ornamental crops (39). Few chemicals can
actually control quackgrass. However, after testing, dichlo-
benil was found to be very effective in quackgrass control
(3,36,39).

Application of Dichlobenil

The method and timing of the application of an herbi-
cide can effect the weed killing properties and can influ-

ence crop injury. Dichlobenil has been effective for quack-grass control without causing injury to established nursery stock and apple trees when applied at 6 pounds of actual ingredient per acre, broadcast on the weed stubble just prior to freeze-up in early winter (36,41,55). Other workers have shown dichlobenil to be an excellent weed control when used at 1 to 6 pounds of actual ingredient per acre and covered (8,12), or incorporated in oak bark (29) or licorice root (22) and mulched to a depth of one inch.

Spring applications of dichlobenil have been used, but often they are not as effective as fall applications (40, 41). Mid-summer applications have had little effect on weed control (3,36,39).

Persistence of Dichlobenil in Soil

The persistence of the chemical is effected by many environmental and edaphic parameters; incorporation with the soil, the type of soil, and the weather conditions (34).

Dana et.al. (16) applied dichlobenil directly to the undisturbed surface of cranberry bogs in the early spring or late fall, and using a bioassay to determine the concentration of dichlobenil, found that four days after the application only 8 percent of the initial level of activity was recovered. They concluded that when the dichlobenil is not incorporated in the soil it has a short persistence due to loss by volatilization.

Miller et.al. (48,49), also working with cranberry bogs, reported that the persistence of dichlobenil was enhanced by overhead irrigation following a surface application of the herbicide.

Sheets et.al. (57) and others (15,16,58) have all worked with soil composition and agreed that the herbicide, being taken up by the organic matter of the soil, was more active in sandy soil than in soil with high organic matter.

Massini (42), using C^{14} labeled dichlobenil, found that potting soil which contained 22 percent organic matter retained all the dichlobenil from the solution. Sand in the same experiment did not retain any dichlobenil.

Sheets et.al. (57) reported a rapid loss of dichlobenil that had been incorporated in soil during the summer months. The rate of loss was rapid until approximately 10 percent of the actual herbicide remained in the soil, and then the loss was considerably slower. It has been shown (3,6), however, that incorporation of the chemical into the soil increased its persistence as compared to surface applications.

Verloop and Nimmo (63), in their latest studies, have shown that dichlobenil decomposed relatively slowly in a saturated sandy soil. After twelve months storage at 20°C, 40 percent of the total dichlobenil was still present.

Movement in the Soil

Horowitz (34) performed some ingenious experiments

showing the area of contamination and the downward leaching patterns of dichlobenil. He reported that dichlobenil leached downward in soil containing 3.3 percent organic matter to a maximum of 3.5 inches when 400 ml of water was dripped on the soil.

Massini (42), using radioactive dichlobenil, found that the herbicide diffused through dry sand to a greater extent than through dry potting soil.

Toxicity: Morphological Effects

Koopman and Daams in 1960 (38) first reported the nature of plant injury caused by dichlobenil. These workers reported the inhibition of bud growth, increased diameter of the meristem, local swelling of the stem's parenchyma, and increased leaf thickness. Since 1960, there have been numerous field plot and laboratory tests to determine the phytotoxicity of dichlobenil on numerous field crops. Barnsley and Rosher (6), working with seed germination and seedlings of several crops, found that dichlobenil caused inhibition of growth when applied as a soil spray at less than one pound per acre.

A partial summary of crop toxicity noted by other workers is presented in the following chart.

Plant species	Description of injury	Herbicide application	Ref.
Azalea	growth reduction	incorp. in peat moss	43
(Azalea cultivars	foliage discoloration	surface	21
	injured	surface	31

Blueberry (Vaccinium)	stunting of growth and retardation and inhibition of stem development	soil incorp.	58
Cotoneaster (Cotoneaster)	marginal tip burn	incorp. in mulch	23,2
Cranberry (Vaccinium)	later blossoming reddish tint to leaves		
	later fruit ripening	surface	18
	smaller fruit size	surface	16
Deutzia (Deutzia gracilis)	tip and marginal scorch	incorp. in licorice root mulch	23
Douglas-fir (Pseudotsuga menziesii)	fresh weight reduction of stems, leaves, and roots - wilting brown cambium	surface	4
Hydrangea (Hydrangea petiolaris)	stunting and leaf discoloration	surface	2
Privet Hedge (Ligustrum)	growth reduction	surface	3
Peach (Amygdalus)	slight interveinal chlorosis larger leaves	surface	32
Pear (Pyrus)	slight edge yellowing of leaves	surface	32

Plus   foliage discoloration   soil incorp.   33

  (Prunus spp.)

Lilac   growth suppression   surface   l

  (Syringa)

Toxicity: Anatomical Effects

     Milborrow (45) has observed blackening and death of
the shoot apical meristem in beans and large amounts of
dark-brown material in the apical meristem, phloem, and
cortical tissues of sugar beets. He also microscopically
examined root tips of germinating oats that were treated
with l ug dichlobenil per l ml of nutrient solution and
found:

     1.   cell division ceased eight hours after application;
           and

     2.   the nuclei and cell walls were found to be quite
           different from the untreated. The nuclei appeared
           more granular and stained less readily, and the
           chromosomes in some cells were mottled. The cell
           walls in the meristem region showed a tendency to
           separate and the middle lamella did not stain so
           heavily with ruthimun red.

     Milborrow (45) also experimented with tomato roots and
noticed that those previously grown in nutrient culture and
then transferred to a solution containing 0.5 ug dichlobenil
per ml exhibited the following:

1. growth stopped;

2. within a few hours a pale brown pigment was formed;

3. within a few hours the characteristic blue-white

    flourescence of healthy roots in screened u-v light

    (360 - 370 nm) disappeared; and

4. within twelve hours the meristematic region swelled

    slightly.

Koopman and Daams (38) reported that dichlobenil caused
the inhibition of bud growth, local swelling of the stem
parenchyma, and increased leaf thickness in oats.

Akobundu (5), working with purple nutsedge (Cyperus
rotundus), observed the destruction of the vascular bundle
and associated parenchyma cells in young tissues and also
changed the distribution of assimilate products.

Ahrens and Leonard (4), working with young Douglas-fir
trees, observed that the roots were more sensitive to dichlo-
benil injury than was the transition zone of the stem.

Modes of Action

Comparison with Boron Deficiency

There has not been any one mode of action that can
account for dichlobenil's herbicidal activity. Milborrow
(45) has suggested that dichlobenil acts in the same manner
as boron deficiency. He reported the findings of Wallace
(1944), Gauch and Duggar (1954), and Whittington (1957),
and others working with mineral deficiency. He compared
the symptoms that these workers noticed with boron deficiency

to the symptoms that he noticed with dichlobenil toxicity.

The similarities of boron deficiency and dichlobenil injury

are: inhibition of shoot and root growth, followed by

browning and death of the meristematic cells; cessation of

cell division; and a higher chlorophyll content in bean

plants.

Effects of Dichlobenil on Oxidative Phosphorylation

Foy and Penner (26), working with mitochondrial fractions

isolated from cotyledons of etiolated cucumbers, reported

that dichlobenil uncoupled oxidative phosphorylation. These

results seem questionable, however, especially since both

Milborrow (45), working with oats, and Wit and VanGenderen

(67), working with yeast cell suspension and with isolated

rat liver mitochondria, did not find inhibition of oxidative

phosphorylation from dichlobenil.

Moreland et.al. (50) found that dichlobenil did not

appreciably affect protein synthesis, whereas several other

herbicides were strongly inhibitory. Devlin and Cunningham

(21) found little direct inhibition of the α-amylose-starch

reaction with dichlobenil.

Translocation of Dichlobenil in the Plant

Massini (42), experimenting with radioactive dichlobenil

on bean plants, found that dichlobenil was taken up by all

organs of the plant and translocated from the roots to the

leaves, but at a slower rate than the water stream.

More recently, Verloop and Nimmo (61), using thin layer
chromatography on silica gel, traced the distribution of
soil-applied dichlobenil in beans. They found that the con-
centration of dichlobenil was greater in the roots than the
stem and the least in the leaves. They also found that 90
percent of the radioactive dichlobenil was lost by evapora-
tion after it was translocated to the leaves, whereas the
concentration of dichlobenil remained constant in the roots.

MATERIALS AND METHODS

Investigations involving placement of dichlobenil to root and stem zones of conifer seedlings were conducted under greenhouse conditions. Three months after treatment the plants were examined for morphological effects, and root and stem cross-sections were made for examination of anatomical effects. The stems of field-established plants were treated at the soil surface level in order to study the likelihood of damage from stem penetration under field conditions.

Greenhouse Study

The evergreen plants used for the greenhouse study were: red pine (Pinus resinosa), two-year seedlings; Norway spruce (Picea abies), three-year seedlings; and American arborvitae (Thuja occidentalis), two-year seedlings and rooted cuttings. The plants were transplanted bare-root to potting soil in five-inch plastic pots, where they were allowed to become established for more than one month before treatments were applied.

Individual treatments consisted of surrounding either the transition zone or a section of the roots to 1.5 inches depth of soil that had been prepared with dichlobenil, 50 percent wettable powder. Concentrations of the herbicide were 0,4,8,12, and 16 parts per million of soil dry weight. This treated soil was a sandy loam soil mixed with equal parts of sand, the resulting mix containing approximately

1.5 percent organic matter. The concentrations in parts per million are related to rates used in field application. Normal field rates of dichlobenil vary from 4 to 8 pounds per acre. Assuming approximately one million pounds of soil per acre 3 inches deep, and no herbicide loss, this is equivalent to from 4 to 8 parts per million 3 inches deep. If the herbicide is restricted to the upper 1.5 inches of soil, the rates of application would result in 8 to 16 parts per million.

Ten plants of the red pine and Norway spruce seedlings were treated with each concentration at the root zone, and ten plants were treated with each concentration at the transition zone. Ten plants of the American arborvitae seedlings and cuttings were treated with each concentration at the transition zone only.

The transition zone was defined as the zone above the first root and extending to the soil surface. The treated soil was restricted to the transition zone by means of a small cup, 1.5 inches high and 2 inches in diameter, which held 40 grams of soil. These small cups were cut from a standard 12-ounce, wax-coated drinking cup. It was necessary to make a slit through and a small notch in the middle of the cup in order that the cups could be placed around the stem. This slit and notch were sealed with masking tape. After the young plants were treated, they were returned to their

original plastic pots with drainage holes. These pots were then placed in a 6 x 6 inch plastic pot without drainage holes. The plants were watered from the bottom to prevent downward leaching of the herbicide.

The procedure for treating the roots was similar to that of the transition-zone treatments. The root zone was defined as the region below the first root, extending to the tip of the last root. The middle 1.5 inches of the root zone was treated.

The purpose of the experiment with the American arborvitae was to compare the sensitivity of seedlings and rooted cuttings to dichlobenil. Therefore, dichlobenil-treated soil was placed around the transition zone of both cuttings and seedlings. The treated soil was the same used for the red pine and the spruce. Instead of using the waxed cups to retain the soil, parafilm "M" was used. The soil was scraped back from the transition zone, thus forming a crater. A slit piece of parafilm "M", 3 x 3 inches, was laid down around the stem conforming to the outline of the crater and the slit was then sealed with melted paraffin. The treated dichlobenil soil was placed on top of the cooled paraffin and covered with vermiculite to retain the moisture. The plants were watered from the bottom as previously described.

After three months, the plants were examined for cambial swelling, cambial browning, and root injury. The top

growth of spruce was estimated by measuring the fresh weight

of new growth. Cross-sections of the transition zone and

the root zone were taken to study the observed symptoms on

a cellular level.

Green beans (Phaseolis vulgaris 'tender green') were

germinated in vermiculite under green house conditions and

treated with soil-incorporated dichlobenil on the surface of

the vermiculite. The dichlobenil concentration was 12 parts

per million. This treatment was applied to 10 to 14 day old

plants and the plants were examined 14 days later.

Anatomical Study:

Representative samples of the control and injured roots

were taken from red pine, Norway spruce, and American arbor-

vitae. The preparation and staining procedures were conducted

according to Johansen (35) and Conn et.al. (14). The roots

were washed in water and then placed in a chrom-acetic

killing fluid, then dehydrated with alcohol. They were then

embedded in paraffin. Slides 12 microns thick were made on a

rotary microtome. The fixed sections were stained in safranin

and fast green.

Field Experiments

Three age groups of red pine trees that were established

in the field were selected to be treated with dichlobenil at

the transition zone. Five-year old trees that had been estab-

lished for one year were located on the north side on Massachu-

sett's Route 10 by mile marker 100 in Northfield, Massachusetts. These plants were growing in loam soil covered with three inches of woodchips. The seven to nine year old trees were located in Orange, Massachusetts, on the south side of Massachusetts Route 2 at the junction of Massachusetts Route 122. These trees were growing in clay-loam soil covered with grass. The 12 to 15 year old trees were located in Erving State Forest on the north side of Massachusetts Route 2. These plants were growing in sandy soil with no cover.

Twenty plants from each age group were selected to be treated on December 11 and 12, 1969. The soil was removed to a 1.5 inch depth from around the stem. A disk of black polyethylene film was laid down. A wire collar was then placed one inch from the stem, and the treated soil of 16 parts per million of dichlobenil was placed between the stem and the wire collar. This was then covered with a second polyethylene disk. These plants were examined on June 1, 1970 and July 17, 1970 for general appearance and browning of the cambium region at the transition zone. Stem chips were removed and examined for cambium discoloration.

RESULTS

Morphological Effects

The observations of red pine seedlings three months
after treatment are shown in Table 1. The root-treated
plants had considerable browning in the cambial region,
and at the high concentrations all of the roots in the
treated zone were killed. The transition zone treated plants
had little browning in the cambium region and minor root
injury. Although the data in Table 1 show a few plants with
brown cambium regions at 4 and 8 parts per million applied
to the transition zone, this is believed to be an artifact
since no browning was found at the two higher concentrations.
Also, three of the control plants showed the same brown
cambium region in the root-treated plants.

The observations of the arborvitae seedlings and rooted
cuttings are shown in Table 2. The exposure of the transi-
tion zone of the rooted cuttings to dichlobenil caused a
high percentage of swelling, whereas similarly treated seed-
lings appeared practically normal.

The observations of spruce seedlings are shown in Table 3.
Stem swelling was observed in all dichlobenil treatments of
spruce. At the lower concentrations, transition-zone treat-
ments produced more plants with stem swelling than did root-
zone treatments. Both root-treated and transition-zone treated
plants had about the same number of plants with injured roots.

Table 1. Symptoms of red pine seedlings treated with varying concentrations of dichlobenil applied either to the root zone or the transition zone.

Concentration of dichlobenil (ppm)	Root zone treated			Transition zone treated		
	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem
0	0	3	0	0	0	0
4	3	7	0	2	2	0
8	8[2]	8	0	2	4	0
12	2[2]	8	0	2	0	0
16	5[2]	8	0	4	0	0

[1]Includes plants having more than 10 percent roots dead

[2]All roots dead within treated zone

Table 2. Symptoms of American arborvitae cuttings and seedlings treated with varying concentrations of dichlobenil at the transition zone

Concentration of dichlobenil (ppm)	Cuttings			Seedlings		
	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem
0	1	0	0	1	0	0
4	0	0	6	0	0	0
8	1	0	8	0	0	0
12	2	0	8	2	0	1
16	2	0	9	0	0	0

[1] Includes plants having more than 10 percent roots dead

Table 3. Symptoms of Norway spruce seedlings treated with varying concentrations of dichlobenil applied either to the root zone or the transition zone

Concentration of dichlobenil (ppm)	Root zone treated			Transition zone treated		
	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem	Number with dead roots[1]	Number with brown cambium region	Number with swollen stem
0	5	0	0	4	0	0
4	2	0	2	1	0	5
8	3	0	3	3	0	6
12	2	0	7	2	0	9
16	6	1	8	5	0	8

[1] Includes plants having more than 10 percent roots dead

The fresh weight of new growth on spruce-treated plants was not significantly different from the control plants. (See Appendix I for spruce fresh weight results)

A comparison of the data in Tables 1, 2 and 3 shows obvious differences among species in their response to dichlobenil treatment. The pine plants showed brown cambial regions but no swelling, whereas the arborvitae and spruce showed transition-zone swelling but no brown cambial regions.

Cross-sections of American arborvitae stems are illustrated in Figures 1 and 2. The stem cross-sections of the cuttings that were treated with 8 to 16 parts per million of dichlobenil had an enlargement of the cortical and the phloem ray parenchyma cells. There also seemed to be an increase in the periderm thickness.

Arborvitae seedling stem cross-sections appeared to be no different from the control, having narrow phloem parenchyma rays and a relatively thin cortex.

The pine and spruce cross-sections of the stem showed no difference between the treated and the non-treated.

Cross-sections of the roots of red pine that received 12 parts per million in the root zone showed a slight disruption of the parenchyma cells associated with vascular bundles (Figures 3 and 4). The xylem appeared normal.

Figure 1. Cross-section of stem of American arborvitae
cutting, untreated, showing regularly shaped phloem,
xylem, phloem parenchyma and cortex cells. x100.

Figure 2. Cross-section of stem of American arborvitae cutting, treated with 16 ppm dichlobenil at transition-zone, showing cortical cell proliferation. x40.

Figure 3. Cross-section of root of red pine, untreated, showing normally structured xylem, phloem and pericycle. x100.

Figure 4. Cross-section of root of red pine, treated with
12 ppm dichlobenil at the root zone, showing phloem ray
and cortical cell collapse. x100.

The root cross-section of the root-treated spruce,
(Figures 5 and 6) displayed an obvious accumulation of darkly
stained unidentified material scattered within the central
cylinder. The amount of this dark matter increased with
increasing dosages of dichlobenil.

Anatomical examinations of arborvitae roots were attempt-
ed. However, the vascular tissue was destroyed either as
a result of the dichlobenil injury or ripped during prepara-
tion. No differences could ben seen in the roots of either
the seedlings or the cuttings.

Because of some difficulty in obtaining definitive slides
of anatomical deformities in the woody plants, it was decided
to examine stems and roots of bean seedlings after treatment
with dichlobenil. Beans are an excellent plant for anatomical
studies due to their rapid growth and soft cell walls. Beans
were grown in the greenhouse by methods previously described.

The morphology of the beans that were treated with
dichlobenil showed a common symptom of a brown hypocotyl.
Many roots of the treated bean plants appeared dead when
harvested. A root was considered to be dead if it was black
and soft; as distinguished from a living root which was white
and firm.

Anatomical examinations of the hypocotyl regions
(Figures 7 and 8) indicated a definite collapse of cortical
tissue. The parenchyma and phloem ajacent to the disrupted

Figure 5. Cross-section of root of Norway spruce, treated with 4 ppm dichlobenil at the root zone, showing a slight accumulation of darkly stained material around pericycle. x40.

Figure 6. Cross-section of roots of Norway spruce, treated
with 16 ppm dichlobenil at the root zone, showing a layer
several cells thick with darkly stained material around
the pericycle. x40.

Figure 7. Cross-section of roots of beans, untreated, showing normally shaped xylem and phloem. x40.
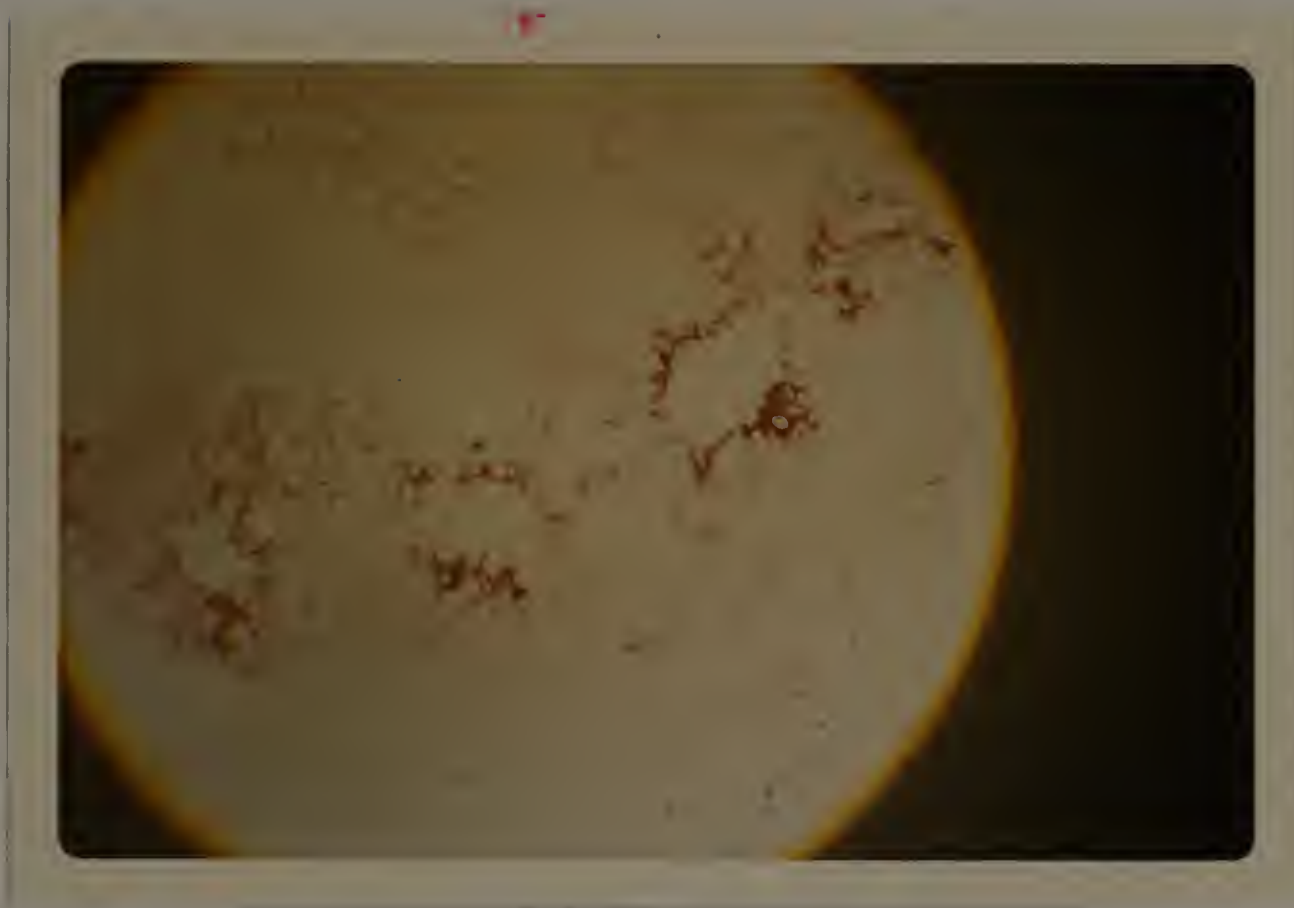
Figure 8. Cross-section of roots of beans, treated with dichlobenil, showing cell collapse in the interfascicular regions. x40.

cortex had an accumulation of darker stain.

Examination of plants in the field plots showed that all the red pine that were treated with dichlobenil at Northfield and Erving State Forest had no visible signs of dichlobenil injury, based on evaluations of gross morphology and samples of the treated transition zone. The treated plants appeared as healthy as the control plants. Unfortunately, the plot at Orange, Massachusetts was a site of vandalism and 40 percent of the plants were missing.

DISCUSSION

The red pine seedlings treated in the greenhouse displayed browning of the cambial region and injury to the roots. The root-treated plants exhibited a higher degree of injury both at the transition zone and to the roots than did the transition-zone treated plants. These results agree closely with those of Ahrens and Leonard (4), who used similar methods with Douglas-fir seedlings. Massini (41) and Verloop and Nimmo (59) found that dichlobenil was taken up faster by the roots than by the stem and was translocated with the water stream. From this it might be expected that more injury would occur at the root zone than at the transition zone.

Dichlobenil did not produce browning in the cambial region on Norway spruce seedlings or American arborvitae cuttings and seedlings.

Based on results of root zone treatments, the roots of the red pine appeared to be more sensitive to dichlobenil than were the roots of Norway spruce. These results also point out that there was an obvious species difference in response to dichlobenil. Furthermore, it was observed that in the transition-zone treatments spruce and arborvitae had stem swelling and no browning and red pine and Douglas-fir (4) had browning but no swelling.

The anatomical cross-section of the arborvitae cuttings

showed enlargement of the cortical cells and of the phello-
derm.   This cell enlargement at the stage that was observed
probably would not be harmful to the growth and development
of the plant.   However, if the proliferation were to be
carried to an extreme, the phloem cells might be crushed and
result in death to the plant.   Plants that exhibited this
swelling obviously had a physiological response to dichlobenil
different from those plants that exhibited cambial region
browning.

It was very unfortunate that the cambial region browning
symptoms were lost in making the cross-sections of the pine
stems.   However, the bean exhibited the same symptoms, and
the cross-sections of the bean stem allowed detailed study
to be made of the cambial browning symptoms.   Destruction of
the cortical cells and some damage to the phloem parenchyma
was noticed in the treated bean stems.   Akobundu et.al. (5)
found that dichlobenil destroyed the young xylem and phloem
tissues in nutsedge tubers, but they did not report any
swelling.   It is suggested that these same tissues may have
been destroyed in the plants showing browning of the cambial
region of red pine in this study and in the Douglas-dir of
Ahrens and Leonard (4).   This browning may be a result of
membrane destruction which would allow the phenols to be
oxidized to the brown pigmented melanin.   This destruction of
cells could lead to eventual death of the plant.

It is suggested that plants which can exhibit the browning response are more sensitive to dichlobenil than plants that exhibit stem swelling.

Anatomical examination of treated pine roots showed disruption of parenchyma and immature phloem cells. Blaser et.al. (9) found that in boron-deficient Thuja plicata plants the phloem cells were less numerous and that the sieve cells were collapsed. He also found that in Thuja plicata roots boron deficiency caused an interruption of differentiation.

Treated spruce roots had a marked accumulation of a dark staining material within the central cylinder. This material may have been a precursor to lignin, a lignin fraction, a lignin by-product, a cell wall fraction, or melanin. Milborrow (45), experimenting with sugar beets, found a similar dark-brown material in the apical meristem, phloem, and cortical tissues. He extracted the pigment in concentrated potassium hydroxide and precipitated by acidification with hydrochloric acid. The precipitate was uneffected by pectinase and insoluble in organic solvents. Judging from the chemical stability, color change in alkali, and solubility characteristics, Milborrow suggested that the dark-brown material may be a melanin.

It is conceivable that dichlobenil binds boron and results in disruption of cell lignification. Parish (53) found that boron deficiency increases peroxidase activity when peroxidase

is attached to the cell wall it is believed to be instrumen-
tal in polymerization of precursors into lignin. Parish
(53) also suggested that boron may facilitate peroxidase
attachment to the cell wall.

The usual field practice is to apply dichlobenil on the
soil surface. This method may result in a high concentration
of the herbicide around the stems, at the soil level, of the
treated trees. From the results of greenhouse experiments,
Ahren and Leonard (4) suggested that Douglas-fir seedlings
could be injured in the field from soil surface application
of dichlobenil without the leaching of the herbicide to root
zones. As a part of this investigation, controlled treatments
were made at the transition zone of red pine seedlings in the
field. The fact that no injury could be found suggests that
transition-zone penetration may not be a problem to field-
established red pine trees at least five years old. It is
also suggested from the greenhouse experiments that red pine
is not as susceptible to injury as Douglas-fir from transition-
zone penetration. The degree of injury reported (4) for
Douglas-fir was much greater than was found here for red pine,
although similar methods were used. Based on either browning
in the cambial region or degree of root kill as criteria for
injury, red pine is more likely to be injured by herbicidal
rates of dichlobenil than Norway spruce or American arborvitae.
Based on stem swelling, American arborvitae rooted cuttings

are more likely to be injured than American arborvitae
seedlings.

These experiments raise many questions that require
further investigations concerning dichlobenil injury, namely:

1. What is the physiological relationship of dichlo-
    benil injury and boron deficiency symptoms?

2. What are the mechanisms that result in cell pro-
    liforation and in cell disruption?

3. Why do arborvitae cuttings respond to a greater
    extent to dichlobenil than do arborvitae seedlings?

4. Why is one species of needled evergreen more suscep-
    tible to dichlobenil than another species of needled
    evergreen?

SUMMARY

In controlled greenhouse studies dichlobenil was
applied at various concentrations in soil layers around
roots or stems of two or three-year old seedlings of red
pine, and Norway spruce, and around the stems of both seed-
lings and rooted cuttings of American arborvitae. The
herbicide was similarly applied around the stem of field-
established red pine trees of three age groups from 5 to
15 years old.

The placement of dichlobenil in the root zone of red
pine caused marked browning in the transition zone,
apparently caused by destruction of cortical cells. Root
treatment of Norway spruce seedlings did not produce brown-
ing, but resulted in swelling in the transition zone, caused
by proliferation of the cortex and the phelloderm. The root
zone treatment of Norway spruce also resulted in the accumu-
lation of a darkly stained material in the pericycle.

The placement of dichlobenil at the transition zone
failed to produce a significant response on red pine, either
on two-year seedlings, or on older plants which were establish-
ed in the fields. On the other hand, placement around the
transition zone of spruce seedlings and arborvitae rooted
cuttings produced a marked swelling response in the treated
area. Arborvitae seedlings did not show response to transi-
tion-zone application.

These results demonstrated species variability in sensitivity to dichlobenil. Furthermore, it is suggested that the browning of the cambial region of red pine and the stem swelling of spruce seedlings and arborvitae rooted cuttings illustrated different physiological responses to the herbicide.

Similarities at the cellular level between dichlobenil injury and boron deficiency are suggested.

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APPENDIX I

Table 1. Fresh wright of new growth of Norway spruce treated with various levels of dichlobenil at the transition zone. Treated 12/29/69 and harvested 4/2/70.

Dichlobenil (ppm)	Fresh weight (gm.)										Mean
0	1.9	2.6	2.0	1.82	6.0	2.8	3.9	6.4	0.7	0.6	2.87
4	4.2	7.8	3.7	3.78	2.7	8.3	4.3	3.0	3.3	4.1	4.52
8	3.65	3.05	5.3	0.9	0.95	5.9	3.8	0.9	5.5	0.1	3.00
12	8.9	3.35	2.6	0.0	2.9	4.8	3.9	7.9	1.7	1.3	3.74
16	2.6	7.0	3.1	3.1	4.15	5.4	5.3	2.8	1.7	5.0	4.02

APPENDIX I

Table 2. Fresh weight of new growth of Norway spruce treated with various levels of dichlobenil at the root zone. Treated 12/29/69 and harvested 4/1/70.

Dichlobenil (ppm)	Fresh weight (gm.)	Mean
0	2.5 2.1 3.95 8.1 1.8 4.0 3.75 2.5 1.8 0.1	3.06
4	3.49 4.92 6.0 3.1 4.15 1.6 3.5 0.9 4.0 3.5	3.52
8	5.3 3.5 1.95 4.26 3.5 2.0 3.65 2.0 3.53 0.82	3.05
12	2.24 6.44 3.2 0.22 1.47 2.5 1.3 2.5 3.11 3.07 1.50	2.51
16	4.84 7.92 1.39 3.0 1.81 5.9 3.4 5.59 1.89 1.70	3.74

APPENDIX I

Analysis of variance of data shown in Appendix I, Tables 1 and 2

Souce	Degrees of Freedom	Sum of Squares	Mean Square	"F" Value
Total	99	387.1427		
Zone	1	5.1484	5.1484	1.310 n.s.
Rate	4	20.3146	5.0786	1.292 n.s.
Z x R	4	7.9906	1.9976	0.508 n.s.
Error	90	353.6891	3.9299	

$Total_{ss}$ $1,544.5711 - 1,157.4284 = 387.1427$

$Zone_{ss}$ $58,128.8401/50 - 1,157.4284 = 5.1484$

$Rate_{ss}$ $23,554.8597/20 - 1,157.4284 = 20.3146$

$Z \times R_{ss}$ $11,908.8201/10 - 1,157.4284 - Zone_{ss} - Rate_{ss} = 7.9906$

$Error_{ss} = Total_{ss} - Zone_{ss} - Rate_{ss} - Z \times R_{ss} = 7.9906$

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