USE OF TWO BENZIMIDAZOLE FUNGICIDES TO IMPROVE ROOT GROWTH POTENTIAL AND FIELD PERFORMANCE OF SHORTLEAF PINE (*PINUS ECHINATA* MILL.) SEEDLINGS

By

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PREFACE

The overall purpose of this experiment was to advance the storage technology through preserving the potential for root growth and field performance of shortleaf pine (Pinus echinata Mill.) seedlings. It was a projection from the preceding works conducted using fungicide chemicals and kaolin clay to improve root growth potential (RGP) and field performance of stored southern pine seedlings. Tests of RGP and field performance of stored shortleaf pine seedlings were performed for 9 root spray treatment combinations of both clay and benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolcarbamate). The 9 combinations were derived from a 3 by 3 factorial of the following clay and benomyl treatments: 1. none, 2. application before and 3. application after storage for 28 days. Similar tests were conducted with mixtures of clay and various concentrations of benomyl and another benzimidazole fungicide chemical, thiophanate-methyl (dimethyl-4,4-O-phenylenebis-3-thioallophanate) before storage. In the RGP test all new roots at least one centimeter long were counted after seedlings were grown for 28 days in greenhouse or growth chamber. Root collar diameter, height above the ground, survival, and survival volume index parameters were measured for field performance evaluation. All data collected were analyzed to supply information on maintenance of RGP and field performance of stored shortleaf pine seedlings. To search for a direct effect of benomyl and thiophanate-methyl on the root elongation, a preliminary test was also conducted using germinating shortleaf pine seeds. Length of the radicle was recorded on a daily basis following the second day after immersion in a fungicide-agar growth medium. Benomyl at 0.5% active ingredient mixed in a clay-slurry was beneficial to RGP of seedlings and their field survival.

Although higher concentration of benomyl seemed to enhance root elongation as a "growth regulator", further investigation is needed to substantiate these finding.

I would like to express my heartfelt gratitude to my major adviser, Dr. Stephen W. Hallgren and, for his remarkable professional guidance, consistent support and encouragement throughout my study in OSU. My heartfelt thanks is extended to Dr. Thomas Hennessey, who also was my adviser for Spring 1993, Dr. Kenneth E. Conway and Dr. Glenn W. Todd for their critical suggestions as members of my Advisory Committee. I would also like to express my sincere thanks to the Oklahoma State University Department of Forestry and Dr. Edwin Miller for their assistance and support when I was seriously in need.

I am always grateful to Dave Ferris, Sr. Research Specialist for the comprehensive knowledge and expertise he offered me for this achievement and beyond. I am also indebted to all staff of forestry and plant pathology laboratory in OSU and at Broken Bow OSU Forestry Research Center for their considerable assistance in laboratory and field work.

My appreciation is due to the Oklahoma State Forestry Nursery in Norman, Oklahoma and Weyerhaeuser Company Nursery in Fort-Towson, Oklahoma for the seeds and seedlings they kindly provided for the entire work.

I wish to express my gratefulness to the people of Ethiopia for the support to this accomplishment.

My special gratitude is also due to Mr. Conrad L. Evans, his colleagues and the Office of International Programs in OSU for their extensive support.

Last but not least, I am glad for this moment to honor my late father Bushra Gudeta for his surviving support to make all the difference. I owe a lot of gratefulness also to my supportive family and compassionate friends.

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CHAPTER I

INTRODUCTION

Shortleaf pine (Pinus echinata Mill.) stands in the interior south of the United States are predominantly natural in origin (Baker 1991). For the purpose of preserving natural shortleaf pine stands and above all to meet the demand for a desired quality product, promotion of artificial regeneration is the prevailing activity so far. The success of artificial regeneration of the stand in the mountainous areas of Ozark and Ouachita National Forest, however, is limited by poor survival and slow growth of seedlings. Inadequate soil moisture content of the sites and poor stock handling and planting techniques are believed to contribute to poor field performances (Brissette and Carlson 1991). Therefore, improved artificial regeneration technology for these and similar areas is an essential path for the sustained productivity of shortleaf pine stands.

Artificial regeneration programs that ought to involve cold storage of seedlings require operations for preserving physiological processes of seedlings that are prone to storage activities. Similarly conditions such as, soil moisture, soil chemical and physical status, and diseases and insect pests, that potentially affect the performance of seedlings have also tremendous importance for successful regeneration programs.

To optimize the productivity of seedlings through minimizing the adverse effects of both storage and field conditions, many studies have been initiated. Studies have also been performed to investigate effects of combinations of factors or agents on the survival and establishment of seedlings.

Shortleaf pine seedling quality, as with other pine species, has often been described in terms of morphology, such as shoot and tap root length, shoot-to-root ratio, root collar diameter, number of lateral roots, and freeness of diseases that are physically conspicuous for grading. However, these are found to be inadequate to appraise seedling field performance especially when it comes to the sites that require long term endurance by the seedling's internal physiology. Therefore, investigation of physiological characteristics and the subsequent improvement of planting performance is essential for successful plantation establishment (Wakeley 1954). Moreover, these would provide benefits to the extent that nursery practices can be modified to generate successful regeneration programs through improved seedling growth and field survival.

Root growth potential (RGP) is one of the most reliable predictors of seedling field performance (Burdett 1987). It is the ability of seedlings to initiate and elongate new roots in an environment favorable for root growth (Brissette and Roberts 1984). This ability is subject to factors that limit plant growth.

Plantation programs which involve frequent and expansive nursery and planting activity require cold storage of seedlings for a period that could have substantial effect on the performance of seedlings in the field. Cold storage is also essential to complete seedlings chilling requirement. Hence, covering of root with clay slurry and fungicides before taking to storage is a routine operation to protect roots from effects of desiccation and diseases in storage (Lantz et al. 1989). To substantiate this procedure, studies have been conducted on the significance to southern pine seedlings which are prone to storage damages. In all of the studies carried out so far, however, results were diverse owing to the inconsistency on the proportion of clay and fungicide applied in a dip or spray and the differences in species of trees.

Apart from the need for information on the level and combination of clay and fungicide to a particular species, investigation on a combined and individual effect of clay and fungicide in connection to application prior and subsequent to storage has not been carried out so far.

An experiment was conducted in the laboratory and under field condition to study: 1) the effect of both kaolin clay and fungicide, benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolcarbamate) on the RGP and field performance of stored shortleaf pine seedlings which emphasized on sorting out the underlying individual and combined effects of fungicide and clay in connection to storage; and 2) the optimum combination rate of kaolin clay with fungicide, benomyl or thiophanate-methyl (dimethyl-4,4-O-phenylenebis-3-thioallophanate), for storage of shortleaf pine seedlings. Data collection for laboratory RGP study was performed in such a way that, after 28 days of greenhouse and growth chamber planting, all freshly elongated roots of at least one centimeter in length were counted from seedlings that had their roots sprayed with: 1) nine randomly assigned treatments from a three by three factorial of clay and benomyl (1. none, 2. before and after storage), and 2) different rates of clay and benomyl or thiophanate-methyl. Parameters such as stem diameter, shoot height, survival and survival volume index were evaluated for field performance of seedlings.

A preliminary test was also conducted on root elongation using both fungicides on germinating shortleaf seeds in the laboratory for the purpose of investigating nonfungitoxic activity of the fungicides.

The overall goal of this study was to promote improved storage technology of shortleaf pine seedlings. The following specific objectives were established:

1. To assess the importance of benomyl and thiophanate-methyl on root growth potential and field performance of stored shortleaf pine seedlings.

2. To investigate individual or combined effects of clay and benomyl on storage of shortleaf pine seedlings.
3. To determine optimum concentration of benomyl and thiophanate-methyl in the clay applied to shortleaf pine seedlings root before storage.

CHAPTER II

LITERATURE REVIEW

Survival of southern pine species in large part depends on the response of seedling quality to the conditions of the planting site. Thus, seedling quality is a fundamental element of the reforestation system through its importance for survival and growth (Feret and Kreh 1985). As the survey conducted on survival of southern pine seedlings indicated there was a general trend of decreasing survival due to factors like poor stock quality, improper nursery practices, poor planting techniques, and unfavorable planting site conditions (Wakeley 1954, Venator 1981, Xydas 1980, Feret and Kreh 1985).

Subsequent to planting, extension of new roots through initiation and elongation of root cells is an early physiological response of seedlings to growth limiting factors in the rhizosphere. This response provides seedlings with favorable water balance by increasing moisture uptake and to match transpiration loss. Therefore, the ability of seedlings to tolerate low soil moisture condition depends upon the potential to develop new root tissue soon after planting (Brissette and Roberts 1984, and Ritchie 1985).

Morphological qualities of seedlings such as, shoot to root ratio, stem height, root collar diameter, size and shape are found to be inadequate to directly explain seedling physiological state at lifting, or after storage and shipment (Feret and Kreh 1985), and also their performance in field (Wakeley 1954, and Burdett 1987). However, under some particular conditions morphological characteristics reflect seedling quality for field performance (Williston 1974, Xydas 1980, Venator 1981, Thompson 1985).

The physiological readiness to rapidly produce new roots and form intimate contact with the soil is termed root growth potential (RGP) (Hellmers 1962, Stone and Jenkinson 1970, and Ritchie and Dunlap 1980). It is measured by new root growth produced when potted seedlings are kept in a greenhouse or growth chamber for a specified time (Brissette and Roberts 1984). Terminologies and methodologies in this field have been inconsistent. In addition to RGP, terms like root growth capacity, root regeneration potential, and root regenerating potential have been used more or less synonymously (Ritchie and Dunlap 1980). Methods for determination of RGP also varies from total length of roots to total number of new roots and total volume or weight of new roots (Ritchie and Dunlap 1980).

Growth of roots is governed by the interaction of internal and external factors. Seasonal variation

(Huberman 1940, Stone and Schubert 1959, Stone et al. 1962, Ritchie 1982), and shoot phenology (DeWald and Feret 1987), nutritional status and growth regulators (Hermann 1977), bud dormancy, (Ritchie and Dunlap 1980) nursery practices and cold storage, (Ritchie and Dunlap 1980, Hallgren and Tauer 1989, Hallgren 1991) influence RGP which ultimately affects field performance of seedlings. In their work Hallgren and Tauer (1989) have also found higher RGP count from shortleaf pine seedlings lifted in December and January and not stored compared to those lifted at the same time and stored for 28 days. However, Brissette and Roberts (1984) have reported a higher RGP in loblolly pine (Pinus taeda L.) seedlings lifted in March than those lifted in November or January and they also noticed higher RGP from large seedlings than medium or small seedlings.

Despite its ultimate effect on stock survival, grading of seedlings on the basis of RGP has not yet been widely adopted due to the result change in due course of the test to accurately indicate the current root growth capacity (Burdett 1987) and the high cost it requires. Extreme environmental conditions such as favorable or unfavorable growing conditions influence the use of RGP to predict field performance (Sutton 1980, Ritchie et al. 1985). In considering these circumstances Burdett (1987) suggested that RGP tests do not predict actual field survival but survival potential.

Storage of seedlings is necessary in areas where production of seedlings is performed on a large scale to satisfy large regeneration programs and also to fulfill seedling chilling requirements. Williston (1974) has reported an acceptable storage time for loblolly pine up to 2 to 3 months. Storage of some southern pine species longer than a few days has been found to result in deterioration of their field performance (Wakeley 1954, Barnett et al. 1988). Moreover, storage was also found to always diminish survival and growth of shortleaf pine seedlings. The effect of storage in this case was more aggravated for late lifted stock (Hallgren and Tauer 1989). On the same species Venator (1985) also reported 26 percent average survival for seedlings lifted in February and stored for 30 days.

Dipping or spraying of southern pine seedlings root with kaolin clay slurry usually improves storeability and field performance (Lantz et al. 1988). Clay slurry helps roots retain their moisture in cold storage, during and after planting which is more significant for moisture stressed location (Bland 1962, Williston 1967, Dierauf and Marler 1969, and Johnston and Ward 1985). It also assists in holding useful substances such as nutrients (Davey 1964) and fungicide chemicals (Lantz 1985) around roots for a long period.

The adverse effect of clay slurry dip on survival of loblolly pine seedlings (Williston 1967, Dierauf and

Marler 1969) and its remarkable improvement with addition of fungicides (Barnett et al. 1988), however, could indicate the ability of clay to harbor fungal disease organisms around roots.

Protection of plants by benzimidazole fungicides, benomyl (Benlate^R 50WP) and thiophanate-methyl (Topsin M^R 46.2F) which have similar antifungal spectrum (Buchenauer et al. 1973) was shown to be due to their systemic activity. Thiophanate-methyl was also considered as an alternative to benomyl considering their similarity in labelling (Chase et al. 1991 and Kaplan 1991) and persistence of their major metabolites, methyl benzimidazole-2-yl carbamate (Jhooty and Singh 1972 and Soeda et al. 1972) or carbendazim (Lyons and Lyda 1987).

Studies on the survival of southern pine seedlings have suggested the importance of mixing benomyl with clay for root treatment before packaging. Improvement of field survival of loblolly pine, slash pine (Pinus elliotti Engelm.), longleaf pine (Pinus palustris Mill.) and shortleaf pine has been observed when this procedure was included in the routine storage practice in the southern pine growing regions (Barnett and Brissete 1988, Barnett et al. 1988). Considerable improvement was noticed on shortleaf and longleaf pine seedlings which are vulnerable to storage damage. A report on the survival of shortleaf pine seedlings that were stored for 3 to 6 weeks with clay-slurry indicated a reduction of 83 to 36% compared to those benomyl-treated seedlings which showed 90% or higher survival (Barnett et al. 1988). Similar improvement was also observed on longleaf pine which was attributed to be due to a systemic protection of benomyl against brown spot disease (Scirrhia acicola [Dearn] (Kais and Barnett 1984, Barnett and Kais 1986, Kais et al. 1986, and Lantz et al. 1988).

In contrast, Stumpff and South (1991) reported the sensitivity of loblolly pine to benomyl and the adverse effect benomyl had on RGP, first-year height growth and survival. Survival of stored loblolly pine was also reduced with application of benomyl on seedlings root with excessive height and high shoot-to-root ratio (Boyer and South 1987).

The fungitoxic activity that benomyl and thiophanate-methyl possess was also found to have a correlation with the activity as a growth regulator on the physiology of plants (Thomas 1974, Beckerson and Ormrod 1986). Activities such as breaking dormancy of wheat seeds (Clark and Scott 1982), promoting growth of soybean callus (Skene 1972), stimulating the germination of celery seeds in the presence of $GA_{4/7}$ (Thomas 1973), extending the storage life of broccoli (Brassica oleracea L. Italica group) (Pressman and Palevitch 1973), and retarding chlorophyll breakdown in excised oat leaves (Staskawicz et al. 1978) have been attributed to the possession a cytokinin-like features by benomyl.

CHAPTER III

MATERIALS AND METHODS

Plant Materials

One-year-old shortleaf pine seedlings were obtained in February 1991 from the Oklahoma Department of Agriculture Forestry Services and in November 1991 and March 1992 from the Weyerhaeuser Company Nursery. In each case the seedlings came from a single open-pollinated family. Following each lifting, seedlings with similar morphological characteristics - shoot height of 15-25cm above the root collar, root collar diameter of 3-6mm and root length of 15-25cm below the collar - were selected before treatment application.

Treatment

Root growth potential, in February 1991, November 1991, and March 1992, and field performance, in November 1991, and March 1992, of shortleaf pine seedlings were evaluated , using kaolin clay and benomyl (Benlate^R 50WP) at the rate of 250 gram dry matter and 0.5% a.i. respectively per liter of water. The clay and benomyl were each applied in three different ways: none(0), before storage(1), and after storage(2). The experimental design was a 3 by 3 factorial of the clay and benomyl treatments to make 9 combinations.

		BENOMYL (0.5% a.i.) 0	1	2
C	0	00	01	02
L	1	10	11	12
A	2	20	21	22
Y				

Except for no treatment (water spray alone) and separate treatment of either benomyl(2.5g/l) or clay(250g/l), the spray combinations had total dry matter of 2.5 gram active ingredient of benomyl and 247.5 gram of clay in a liter of water. In view of the common practices for southern pine seedlings storage, root spray with clay slurry before storage was considered as a standard.

Effects of benomyl (Benlate^R 25WP or 50WP) and thiophanate-methyl (Topsin M^R 46.2WP) added to the clay slurry at different concentration were also tested on RGP and field performance under three lifting periods. Seedlings lifted in February 1991 were treated with five levels benomyl (Benlate^R 25WP) for greenhouse and growth chamber RGP test. In the next two lifting times, November 1991 and March 1992, tests were conducted in a greenhouse and under field conditions with five levels of each of benomyl (Benlate^R 50WP) and thiophanate-methyl (Topsin M^R 46.2F).

The amount of commercial fungicides and clay were used by using the following equation with treatment concentrations in Table 1.

$$\text{Amount of Commercial Fungicide (g/l)} = \frac{\text{Treatment Concentration (\% a.i.)} * \text{(250 g/L Total Dry Matter)}}{\text{Commercial Fungicide (\% a.i.)}} .$$

The treatment mixtures were prepared and applied with a hand-pump type garden sprayer immediately before and after storage. Seedlings were stored in tightly wrapped kraft-polyethylene bags for 28 days at 4^{O}C. Seedlings receiving treatment without clay were sprayed with water alone or the appropriate water plus fungicide mixture and stored with sterile absorbent cotton.

RGP Test

For the RGP test, three seedlings from a treatment in a block were planted into 1 L milk carton pot filled with a 1:1 peat-vermiculite mixture. In February 1991 fifteen blocks (arranged in a randomized complete block design) were placed in a greenhouse and fifteen in growth chamber. In November 1991 and March 1992 RGP tests were conducted only in the greenhouse with fifteen blocks.

After 28 days in the greenhouse and growth chamber, all seedlings were transferred to 4^{O}C temperature cold room before new root count was started. For RGP assessment, roots of seedlings were carefully washed and all freshly

TABLE 1

CONCENTRATION OF KAOLIN CLAY SLURRY-FUNGICIDES MIXED IN 1 L OF WATER

Treatment (a.i. %)	Clay (g)	Commercial Fungicide(g)
February 1991		
		Benlate[R] 25WP
0	250.00	0.00
0.005	249.95	0.05
0.05	249.50	0.50
0.5	245.00	5.00
5.0	200.00	50.00
November 1991 and March 1992		
		Benlate[R] 50WP
0	250.00	0.00
0.125	249.37	0.63
0.25	248.75	1.25
0.5	247.50	2.50
1.0	245.00	5.00
		Topsin M[R] 46.2F
0	250.00	0.00
0.125	249.32	0.68
0.25	248.65	1.35
0.5	247.30	2.70
1.0	244.59	5.41

elongated roots greater than 1 cm in length were counted.

Greenhouse day and night temperature and relative humidity (RH) were recorded using a hygrothermograph. Average day and night temperatures during each experiment were in the ranges of 20-30^{o}C and 10-20^{o}C respectively, and the ranges for day and night relative humidity were 30-60% and 40-90% respectively. Temperatures in the growth chamber with 16 hours of day and 8 hours of night were 25 and 15^{o}C.

Field Test

Concurrent to the RGP test for November 1991 and March 1992 lifting dates, the remaining portion of seedlings were field planted at Broken Bow OSU Forestry Research Center using randomized complete block design with 10 blocks. Each treatment had a 10-tree row plot in each block planted with 50 x 50 cm^2 spacing. Herbicide and insecticide were applied to control weed competition and insect damage. Information on annual precipitation and evaporation conditions of the research center was obtained from the nearby weather station (APPENDIX B, Figure 1) .

Parameters for assessment of field planted seedlings included survival, diameter at the base of the stem, seedling height above the ground, and survival-volume index (SVI). Field data on the parameters were collected from October 17 through 22, 1992 and survival-volume index

(Tuttle et al. 1988) was estimated using the following ratio:

$$\text{SVI} = \frac{\text{Sum of volumes of live seedlings}}{\text{Number of planted seedlings}}$$

Root Elongation Test

Shortleaf pine seeds from family 138-1 were obtained from the State of Oklahoma Department of Agriculture, Forest Services. After 24 hours soaking in aerated water, seeds were stratified in polyethylene bag at 4^{o}C for about 60 days. Afterwards seeds were surface sterilized by soaking in 10% bleach for one minute before placing in a germinator at 22^{o}C. Germinating seeds that had similar radicle length were separated into four categories or blocks. One germinating seed from each category or block was planted on a treatment-water agar mix growth medium prepared by a technique for in vitro root observation (Russell, 1987). The test was conducted with 0, 1, 5, 10, 50, and 100ppm a.i. treatment levels of each of benomyl and thiophanate-methyl fungicide. Data on increase in length of the radicle were collected every other day beginning on the second day after planting.

Data Analysis

Analyses of variance (ANOVA) was used to test for the significance of treatment effect. The least significance difference (LSD) was calculated at the 5 percent level to test for differences among treatment means (Steel and Torrie 1980).

CHAPTER IV

RESULTS

Effect of Kaolin Clay and Fungicide Root Treatment

RGP

RGP varied widely among lift dates. The overall mean RGP count was 105 for February 1991, 7 for November 1991 and 40 for March 1992. RGP was nearly equal in the greenhouse and growth chamber tests of February 1991. Besides, RGP was higher from seedlings that were treated with benomyl after storage and tested in a growth chamber (APPENDIX A, Table 1) than those tested in greenhouse (APPENDIX A, Table 2).

RGP was increased 50 to 60 percent by applying benomyl to the roots before storage regardless of whether a clay slurry was applied in the greenhouse test in November 1991 (Figure 1; APPENDIX A, Table 3). When benomyl was applied after storage RGP of seedlings was not different than seedlings that did not get benomyl treatment.

In the March 1992 test both benomyl and clay treatments showed large effects and there was a slight tendency for the treatments to interact in their effect on RGP (APPENDIX A, Table 4). Benomyl applied before

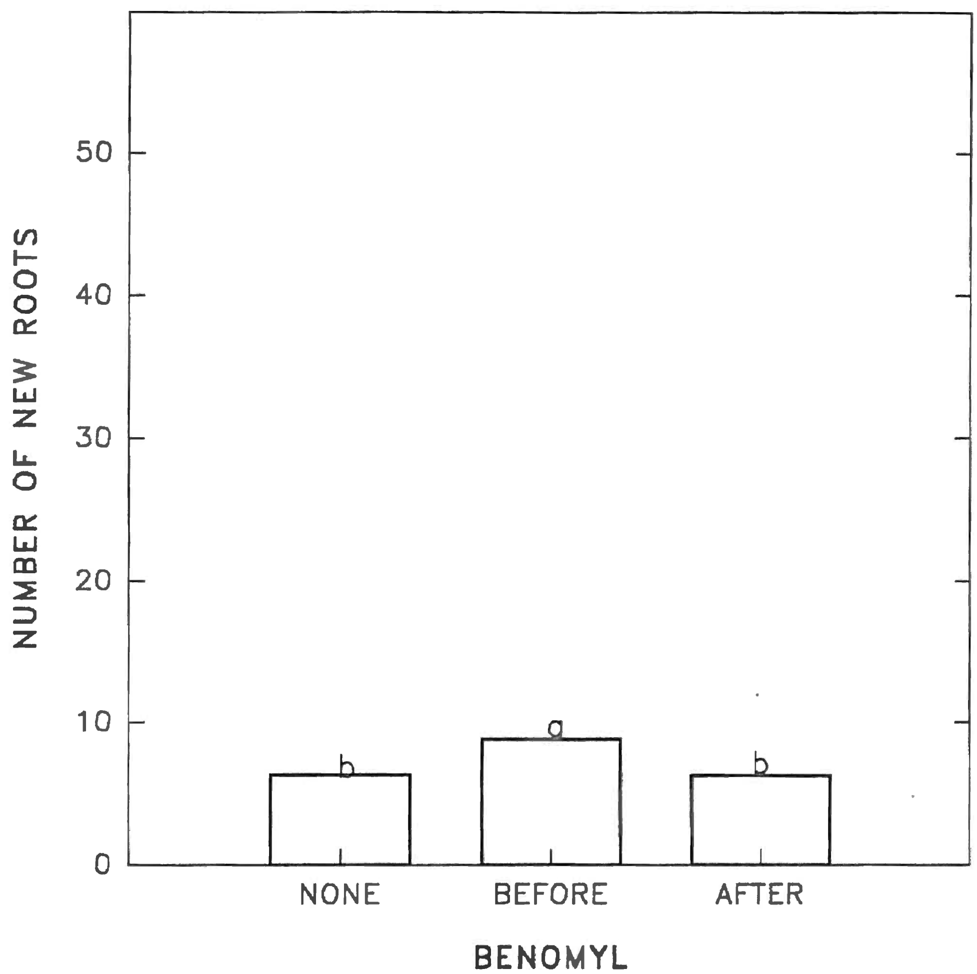

Figure 1. Effect of benomyl treatment (0.5% a.i.) on RGP (number of new roots) of shortleaf pine seedlings lifted in November 1991 and stored for 28 days before testing. Letters indicate means different at the 5 percent level. n=15

storage increased RGP 60 percent over the other treatments regardless of the clay treatment (Figure 2) and clay applied before storage increased RGP an average of 30 percent over the other treatments (Figure 3). However, the effect of clay was somewhat different for each benomyl treatment (Figure 4). When benomyl was applied after storage, RGP was not different among the clay treatments. The greatest RGP was obtained by seedlings treated with benomyl before storage and without clay. When no benomyl was applied the greatest RGP was also shown by seedlings that received clay before storage.

Field Performance

Benomyl treatment before storage increased survival (Figure 5; APPENDIX A, Table 5), shoot height (Figure 6; APPENDIX A, Table 7) and survival volume index (Figure 7; APPENDIX A, Table 9) of November 1991 lifted seedlings as compared to treatments of no benomyl or benomyl after storage. In contrast, clay treatment did not show significant effect on any field performance parameters.

Except for survival which was increased due to benomyl treatment before storage, (Figure 8, and APPENDIX A, Table 6), performance of March 1992 lifted seedlings were not affected by any one of the treatment combinations (APPENDIX A, Table 8 and 10).

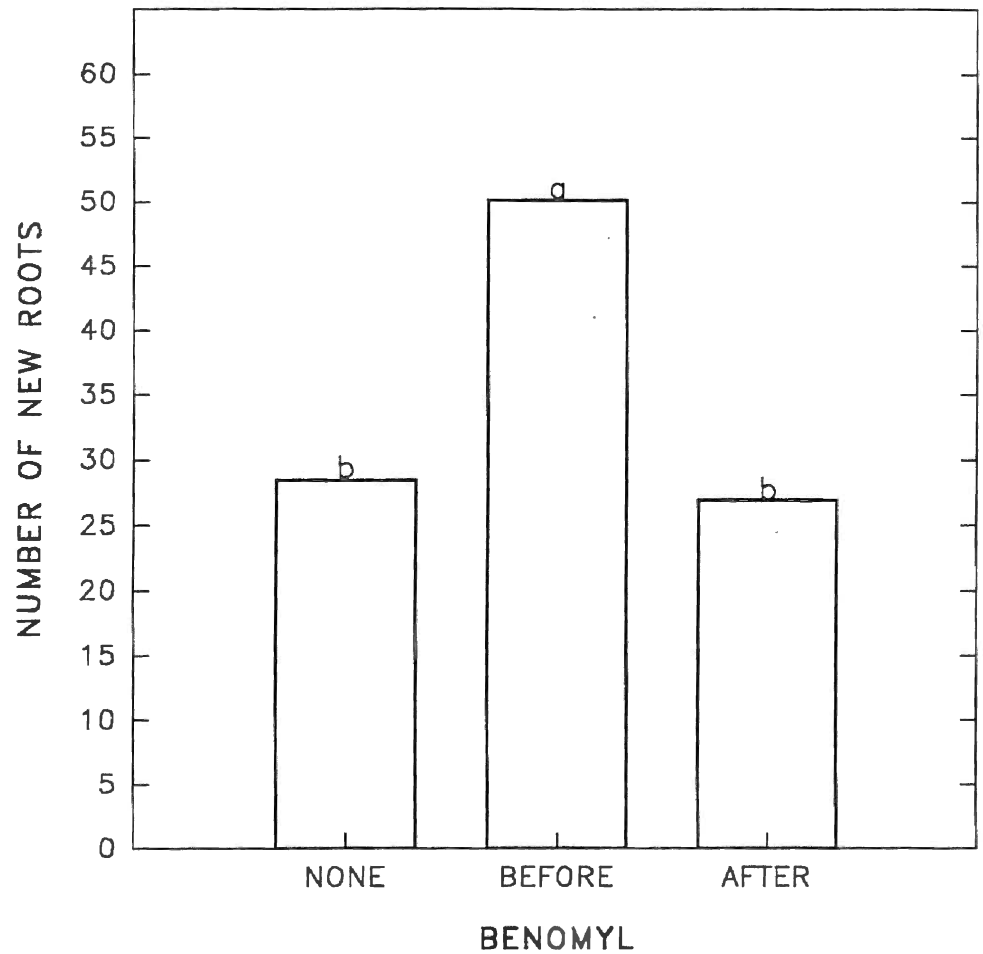

Figure 2. Effect of benomyl treatment (0.5% a.i.) on RGP (number of new roots) of shortleaf pine seedlings lifted in March 1992 and stored for 28 days before testing. Letters indicate means different at the 5 percent level. n=15

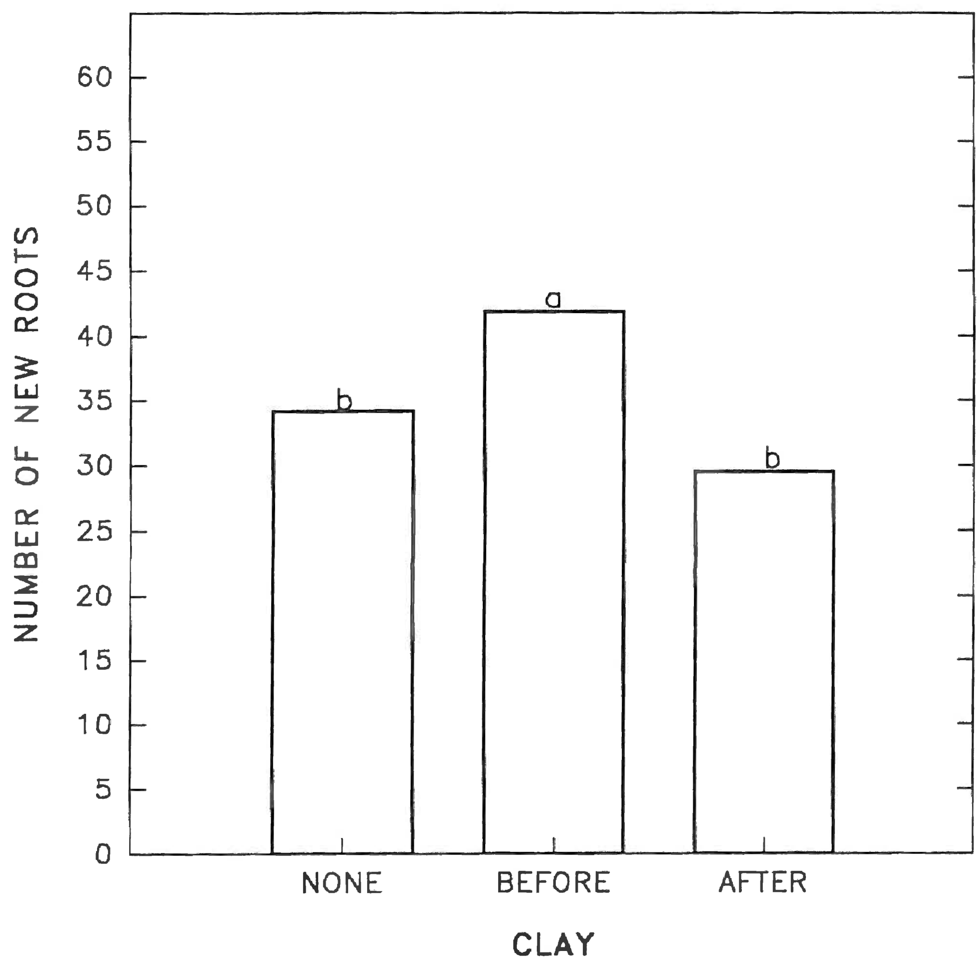

Figure 3. Effect of clay treatment on RGP (number of new roots) of shortleaf pine seedlings lifted in March 1992 and stored for 28 days before testing. Letters indicate means different at the 5 percent level. n=15

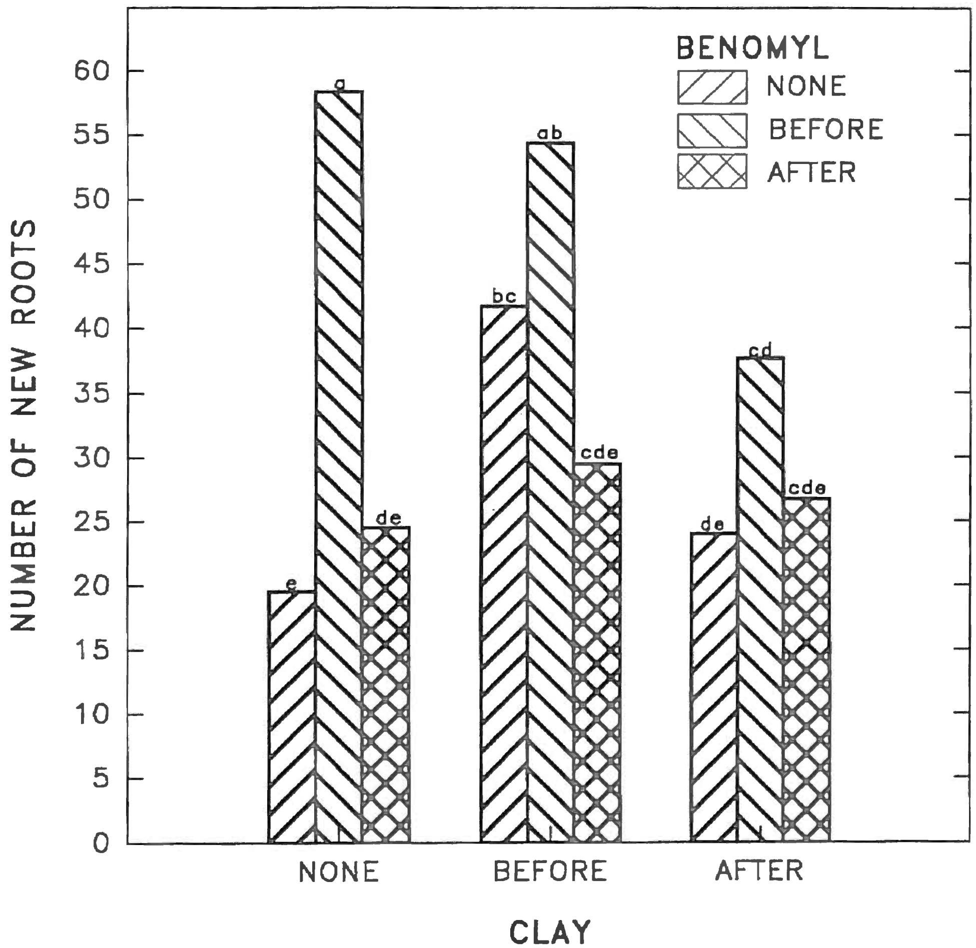

Figure 4. Effect of benomyl (0.5% a.i.) and clay on RGP (number of new roots) of shortleaf pine seedlings lifted in March 1992 and stored for 28 days before testing. Letters indicate means different at the 5 percent level. n=15

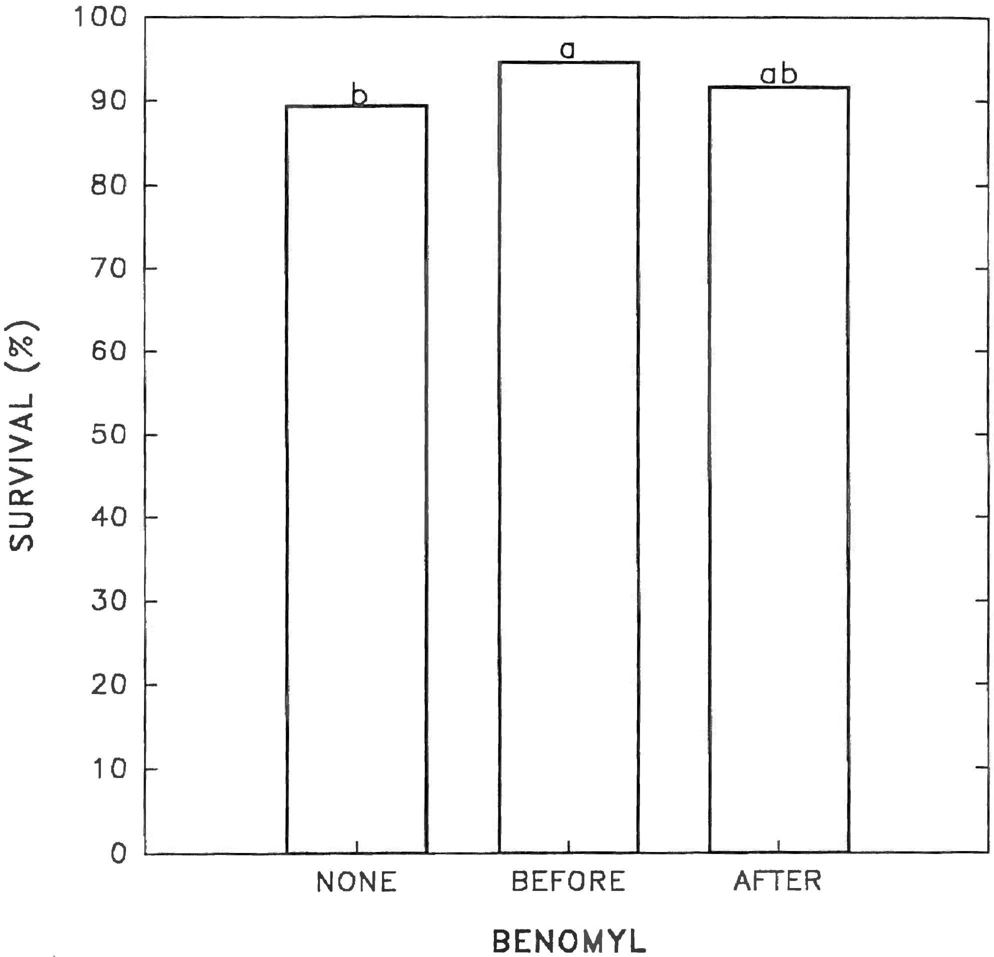

Figure 5. Effect of benomyl treatment (0.5% a.i.) on survival of shortleaf pine seedlings lifted in November 1991 and stored for 28 days before field planting. Letters indicate which means are different at 5 percent level. n=10

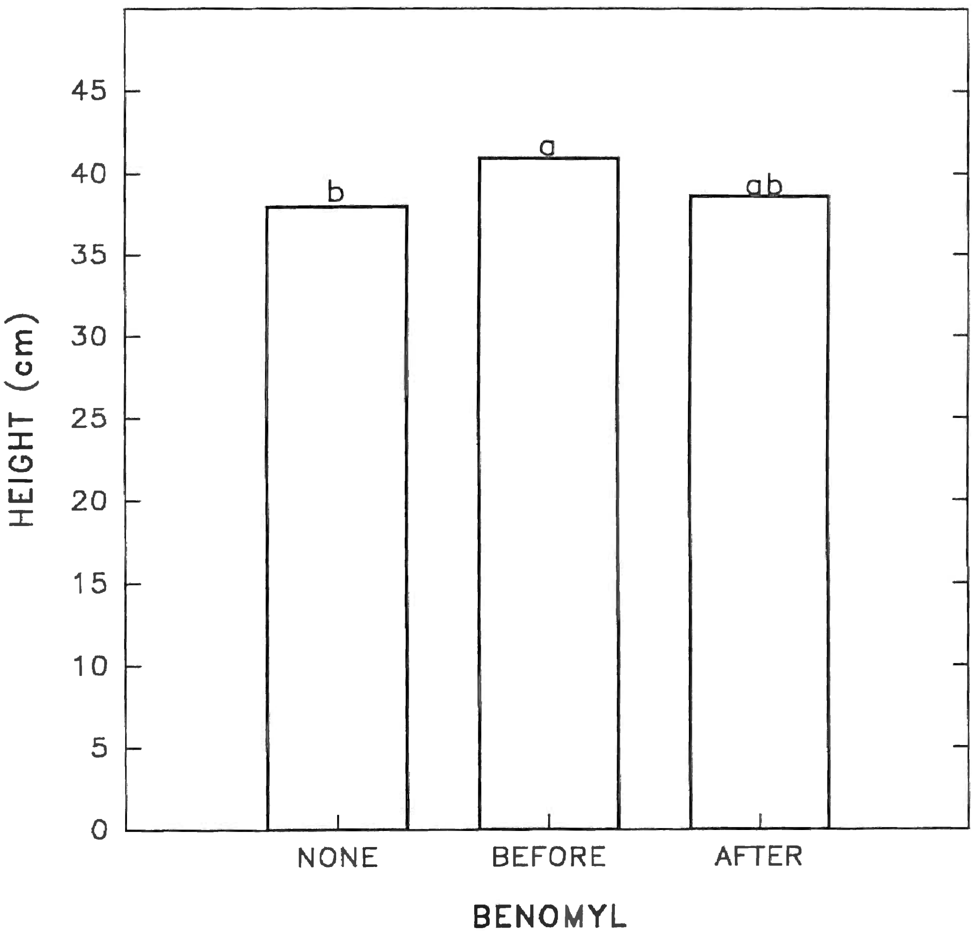

Figure 6. Effect of benomyl treatment (0.5% a.i.) on height of shortleaf pine seedlings lifted in November 1991 and stored for 28 days before field planting. Letters indicate which means are different at 5 percent level. n=10

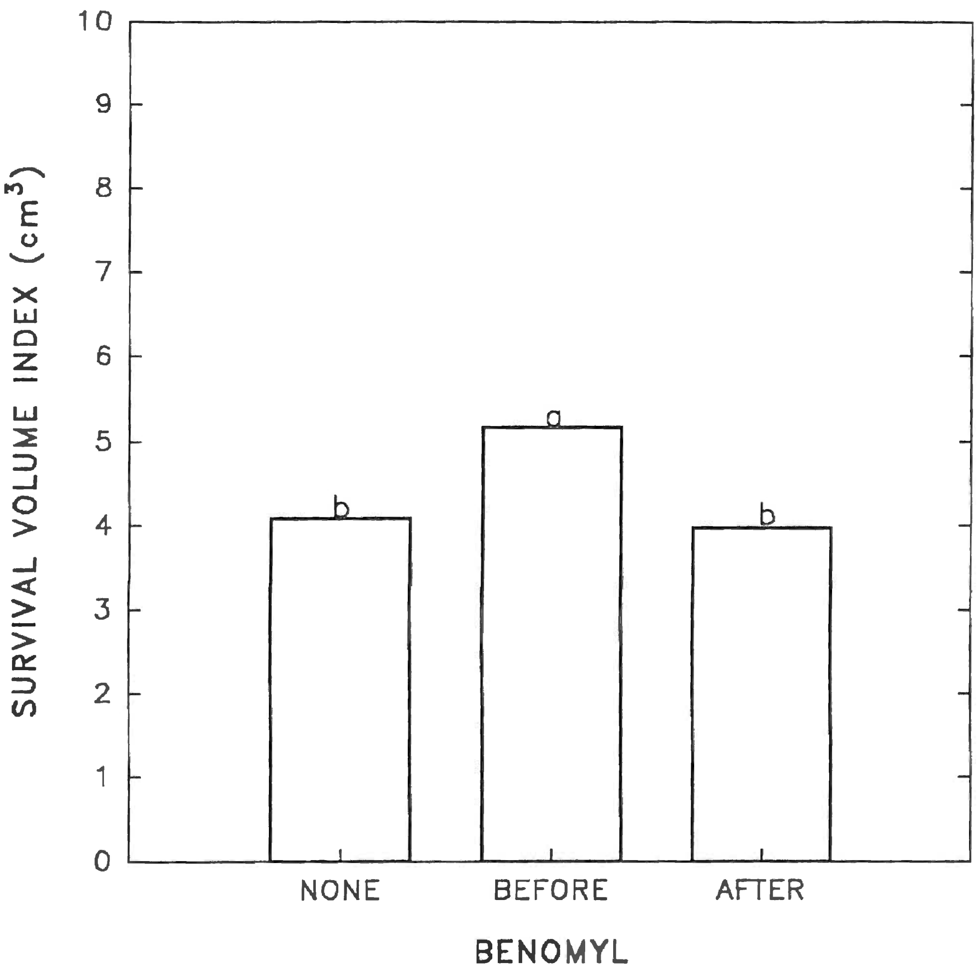

Figure 7. Effect of benomyl treatment (0.5% a.i.) on survival-volume index of shortleaf pine seedlings lifted in November 1991 and stored for 28 days before field planting. Letters indicate which means are different at 5 percent level. n=10

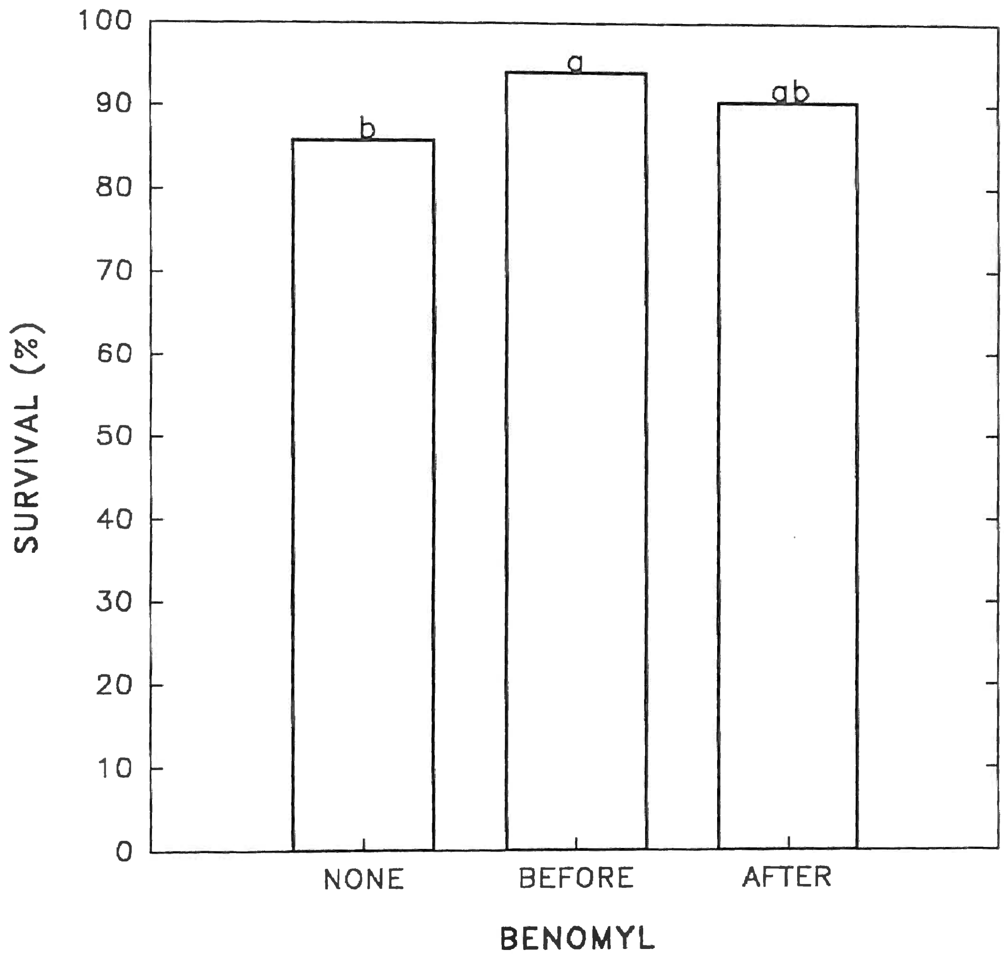

Figure 8. Effect of benomyl treatment (0.5% a.i.) on survival of shortleaf pine seedlings lifted in March 1992 and stored for 28 days before field planting. Letters indicate which means are different at 5 percent level. n=10

Effect of Fungicide Type and Concentration

RGP

February 1991 lifted seedlings had higher number of new roots in the growth chamber when their root was sprayed with mixture of clay slurry and benomyl at 0.5 percent active ingredient level (Figure 9; APPENDIX A, Table 11). Greenhouse tested seedlings had also higher number of new root for the same treatment level of benomyl (Figure 10; APPENDIX A, Table 12). Concentrations lower and higher than 0.5 percent active ingredient of benomyl used at this period resulted in reduced RGP stored seedlings.

RGP of November 1992 lifted seedlings was highest for the one percent active ingredient of benomyl (Figure 11; APPENDIX A, Table 13). Along with the remaining levels of benomyl, all levels of thiophanate-methyl did not improve RGP of stored seedlings.

March 1992 lifted seedlings had higher RGP with 0.5 percent active ingredient of benomyl than the rest levels of benomyl and all levels of thiophanate-methyl (Figure 12; APPENDIX A, Table 14).

Field Performance

Excepting SVI of November 1991 lifted seedlings which was altered due to fungicides level differences (Figure 13; APPENDIX A, Table 17), field performances

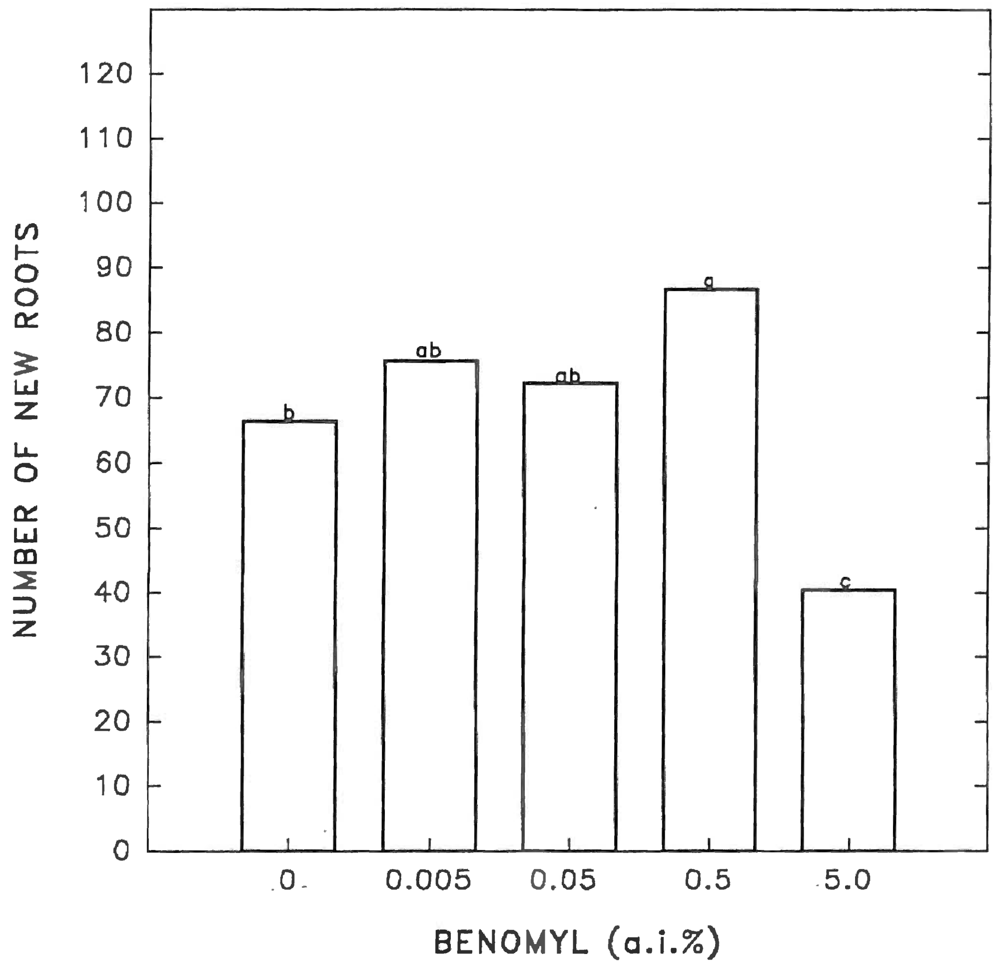

Figure 9. Effect of benomyl concentration on RGP (number of new roots) of shortleaf pine seedlings lifted in February 1991 and stored for 28 days before testing in a growth chamber. Letters indicate means different at the 5 percent level. n=15

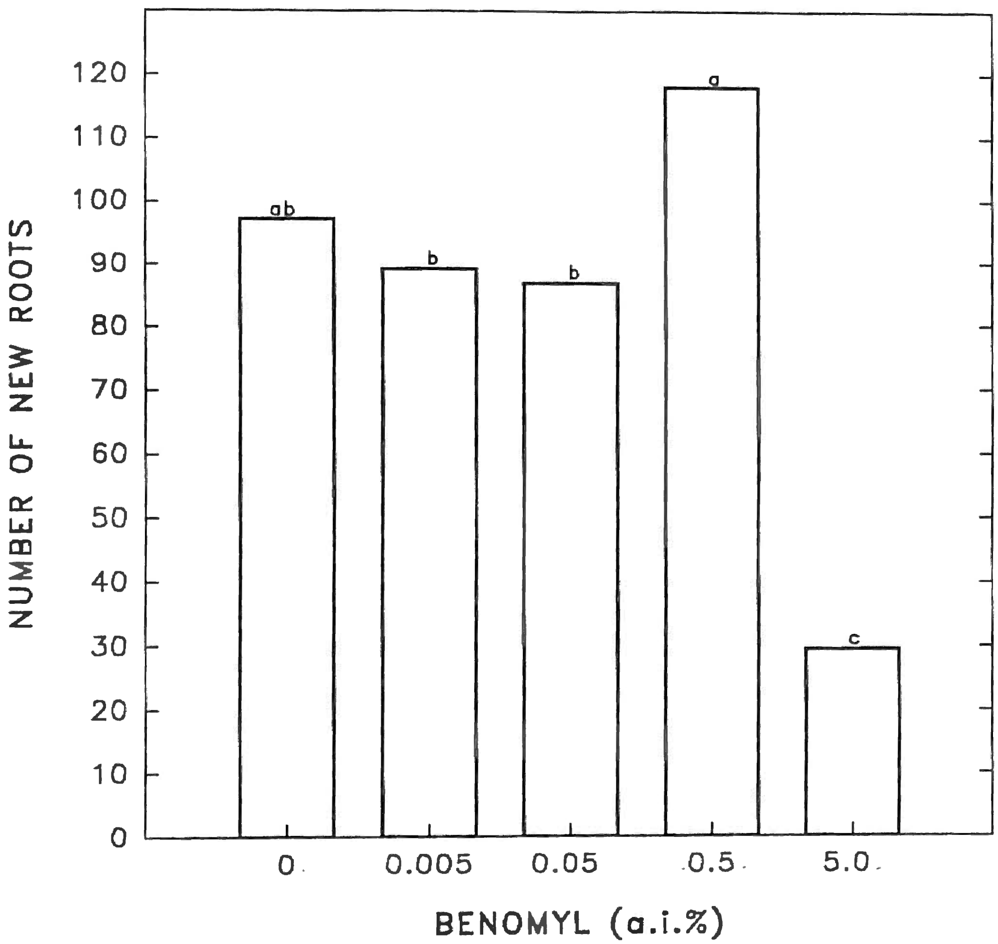

Figure 10. Effect of benomyl concentration on RGP (number of new roots) of shortleaf pine seedlings lifted in February 1991 and stored for 28 days before testing in a greenhouse. Letters indicate means different at the 5 percent level. n=15

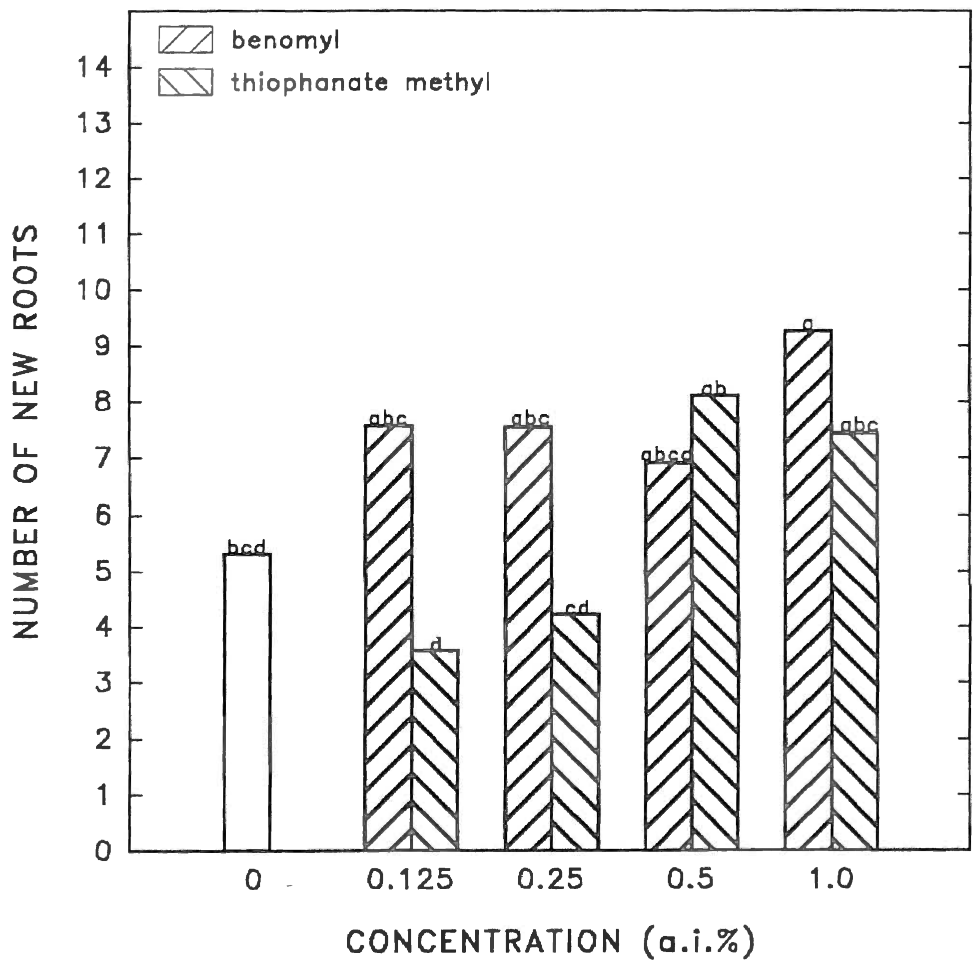

Figure 11. Effect of fungicide type and concentration on RGP (number of new roots) of shortleaf pine seedlings lifted in November 1991, stored for 28 days and tested in a greenhouse. Letters indicate which means are different at the 5 percent level. n=15

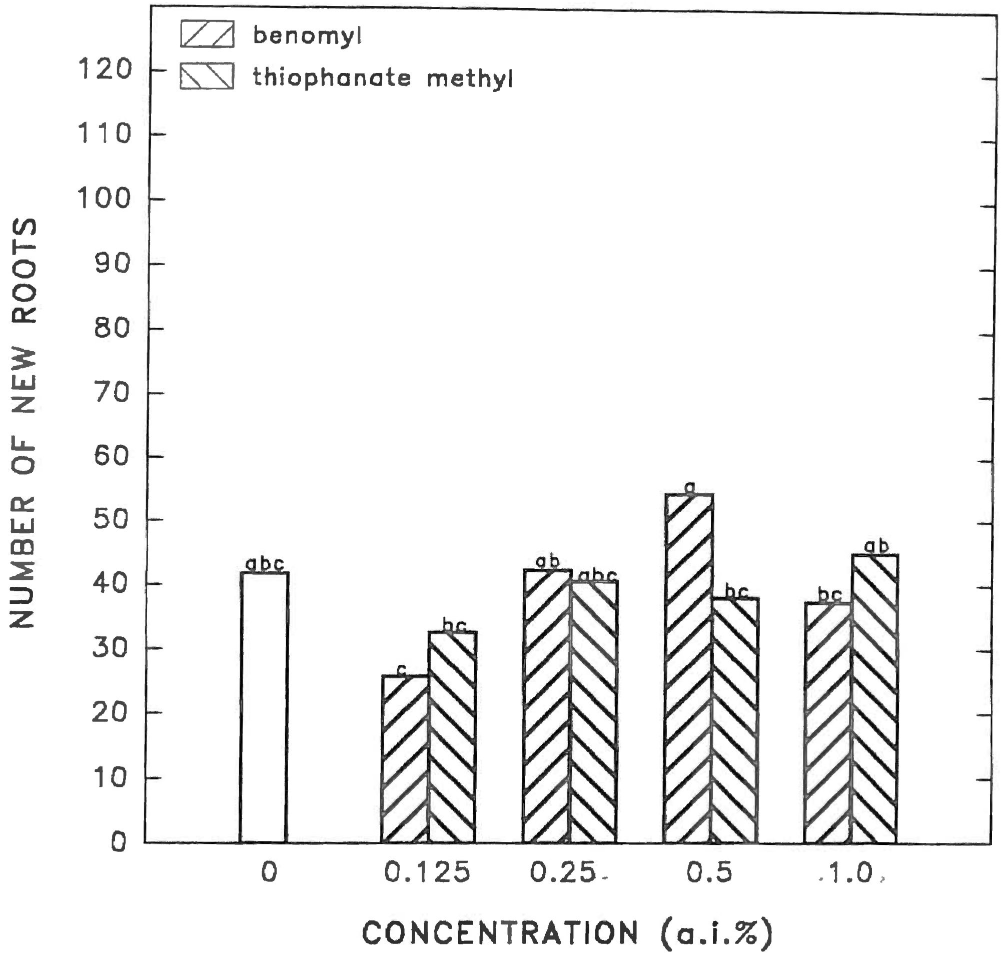

Figure 12. Effect of fungicide type and concentration on RGP of shortleaf pine seedlings lifted in March 1992, stored for 28 days and tested in a greenhouse. Letters indicate which means are different at the 5 percent level. n=15

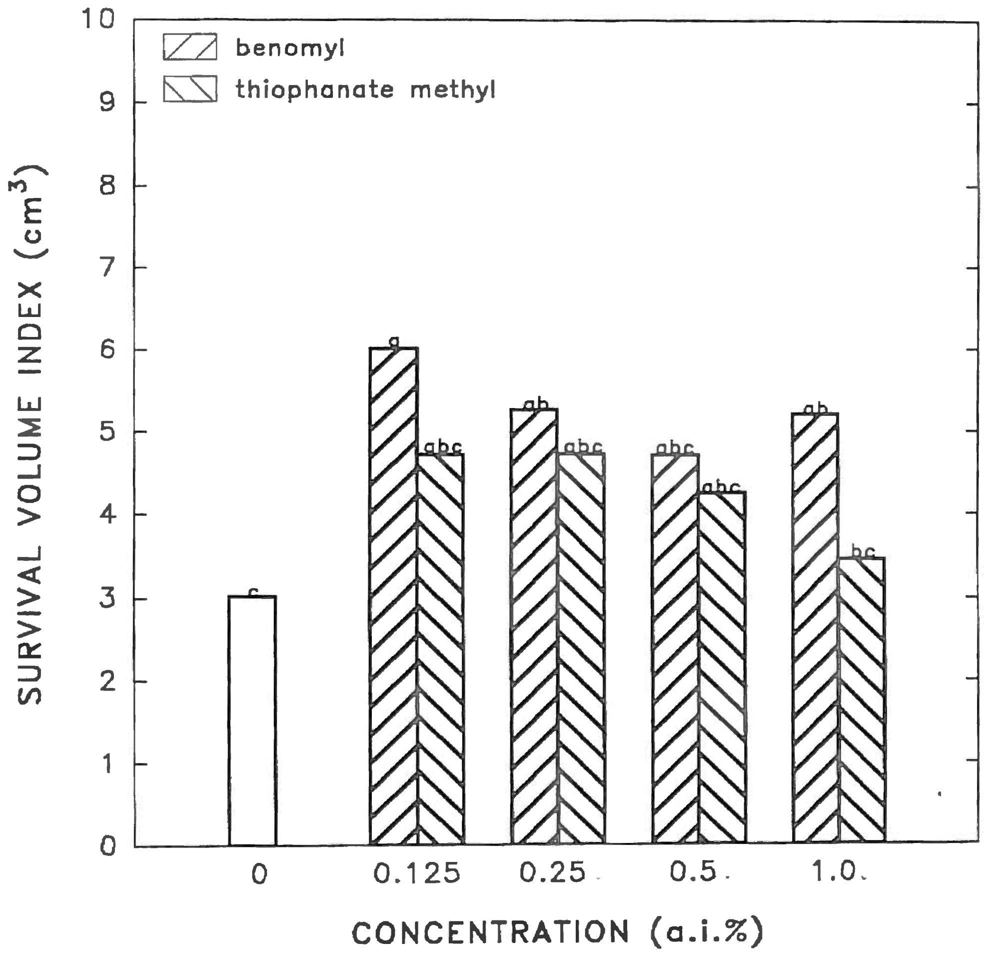

Figure 13. Effect of type and concentration of fungicides on survival-volume index of shortleaf pine seedlings lifted in November 1991 and stored for 28 days before planting. Letters show mean values that are different at 5 percent level. n=10

with regards to root collar diameter, shoot height, and field survival were not statistically different from the untreated seedlings. All seedlings that were treated with 0.125, 0.25, and 1.0 percent active ingredient of benomyl had higher SVI than seedlings untreated with benomyl. Seedlings tested with all levels of thiophanate-methyl, however, did not have significant change on their SVI than the control.

The survival of seedlings lifted in March 1992 was significantly improved with benomyl at both 0.5 and 1 percent active ingredient level and with thiophanate-methyl at the level of 0.5 percent active ingredient (Figure 14; APPENDIX A, Table 16).

Root Elongation

The result regarding root elongation experiment was obtained from the analysis conducted on the fourth day of the test when differences were at the stage for statistical analysis. Due to some missing values the analysis was made using general linear model. It was found that benomyl at the concentration levels studied was not as effective as thiophanate-methyl was at 5.0 parts per million to cause an increase in the radicle length of germinating seeds (Figure 15; APPENDIX A, Table 20).

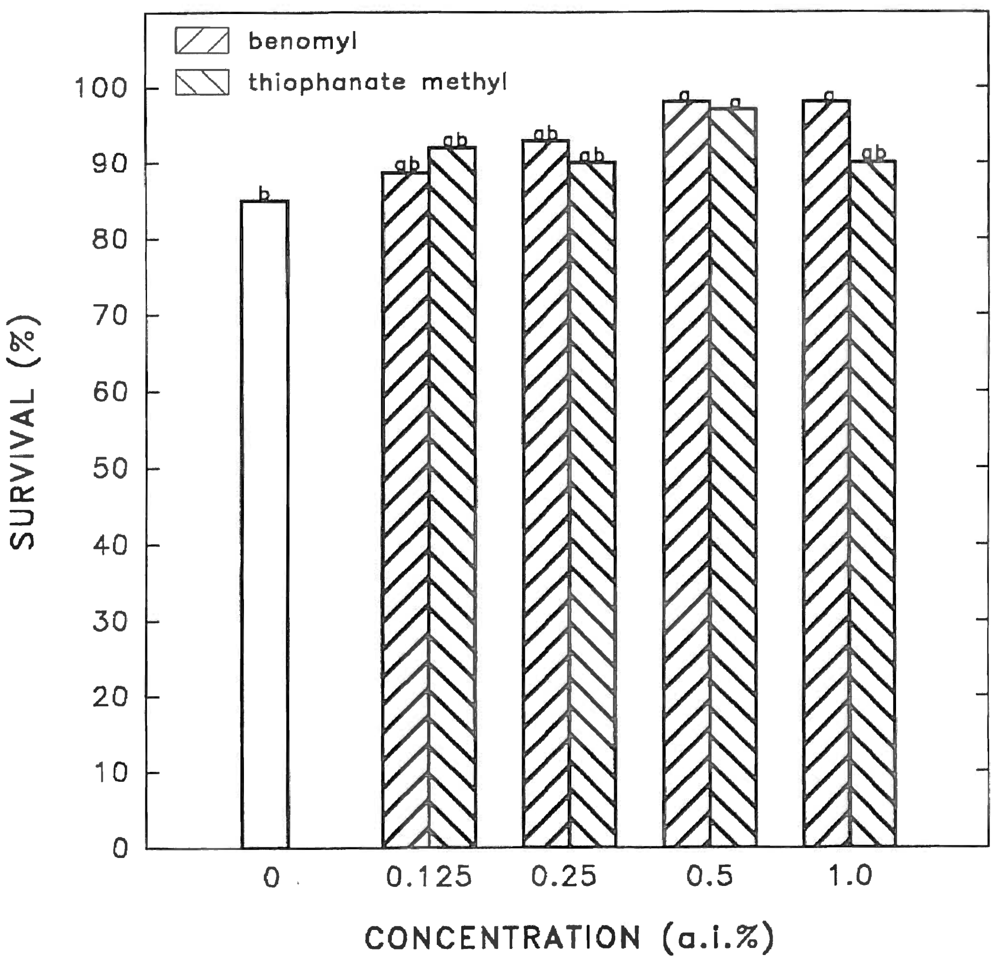

Figure 14. Effect of fungicide type and concentration on survival of shortleaf pine seedlings lifted in March 1992, stored for 28 days and tested in a greenhouse. Letters indicate which means are different at the 5 percent level. n=10

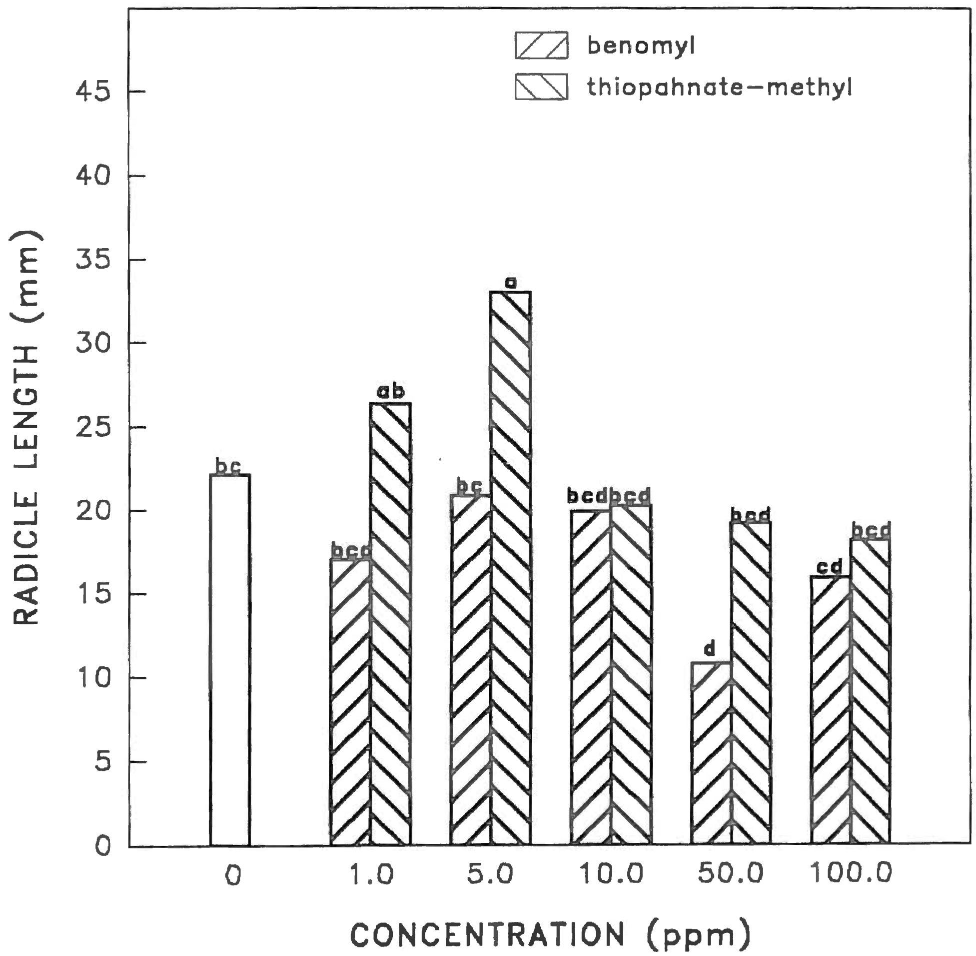

Figure 15. Effect of fungicide type and concentration on root elongation of germinating shortleaf pine seeds tested in a laboratory. Letters indicate which means are different at 5 percent level. n=4

CHAPTER V

DISCUSSION

Root growth potential and field performance of seedlings, also respectively referred to as physical and material attributes to the quality of seedlings (Ritchie 1985) depend on physical, chemical and biological factors that limit growth and development at any seedling developmental stage. Seedling quality with respect to roots is primarily connected to the capacity to maintain favorable water balance through offsetting the loss by transpiring parts. Hence, the potential for rapid extension of new roots in the field promotes initial survival and facilitates first-year establishment of seedlings (Wakeley 1954).

Hellmers (1962) stated that carbohydrate in the root is a good indicator of a seedling's potential for performance since it is the last to be depleted. This potential has also been described as having a pattern influenced by the seasons (Huberman 1940, Stone and Schubert 1959, Stone et al. 1962, Ritchie 1982). In general seedlings lifted in mid-winter tend to have higher RGP which even increase following storage, than fall- or spring-lifted seedlings. This rhythm seems to be related to the bud dormancy cycle, and also reflects internal carbohydrate availability and its allocation priorities (Ritchie and Dunlap 1980). In this current experiment, seedlings lifted in February 1991 had more new root growth in the greenhouse than March 1992 lifted seedlings which had more new roots than seedlings lifted in November 1991. The variation in the new root counts among seedlings lifted from the same nursery bed in November 1991 and March 1992 appeared to correlate with the suggestion that RGP buildup in winter coincides with the accumulation of chilling hours and culminates with the fulfillment of the chilling requirement. It is also consistent with the previous finding of the strong effect of lift date on RGP and field survival of shortleaf pine seedlings (Hallgren and Tauer 1989).

Duration of storage likewise affects bud dormancy and carbohydrate reserves of the seedlings. Storage conditions that are less conducive in terms of weather as well as longer storage periods are critical factors to influence performance and outplanting survival of seedlings.

Root treatment with fungicides such as benomyl mixed in a clay slurry has been found to preserve RGP of stored seedlings (Kais and Barnett 1984, Barnett and Kais 1986, Barnett and Brissette 1988). Although the advantage of useing clay was to protect seedlings from desiccation and for uniform distribution of the fungicides (Bland 1962, Dierauf and Marler 1969), in the present experiment its effect was inconsistent throughout lifting times and at times less significant than with benomyl to improve RGP of seedlings. Root growth potential, however, was preserved by applying benomyl at 0.5 percent active ingredient before storage regardless of clay application. Among the parameters assessed, field survival of November and March lifted seedlings was improved by spraying benomyl before storage. Height and survival volume index of November lifted seedlings were also improved with benomyl treatment before storage. This indicated that there was inadequate interaction of benomyl and clay to affect RGP and field performance of seedlings. Application of clay alone was not found to be detrimental to RGP or field performance of seedlings which was not the case with other findings. For instance, Barnett et al. (1988) did find reduced first-year survival from longleaf and shortleaf pines seedlings that were treated with clay alone than seedlings treated with clay-benomyl mixture at the time of packing. They also found a reduced 3-month field survival of loblolly and slash pines seedlings due to treatment with clay than with clay-benomyl mixture before storage.

Inasmuch as the protection that clay may provide to roots against desiccation, it may enhance the development of diseases on roots if seedlings are from nurseries with disease infected seed beds. Increased temperature and humidity in the storage could also contribute to the development of disease. Clay when applied after cold storage, i.e. when seedlings are to resume growth, could also have an effect on RGP (Figure 4) through its effect on strong adsorption of benomyl.

The interaction effect of clay and benomyl was meager so that the benefit of benomyl to improve RGP was not due to its effect on pathogenic fungi that could be encouraged by clay. In most preceding investigations, however, the importance of benomyl was emphasized because of its toxicity to fungal diseases in storages. It was found that benomyl improves RGP of southern pine species with low storeability by acting against storage diseases and other fungi antagonistic to mycorrhizal association. Barnett et al. (1988) reported that seedlings of longleaf and shortleaf pines stored for 3 or 6 weeks period were better in first-year survival when their roots were treated with kaolin clay and benomyl at 5 percent active ingredient before storage. RGP of loblolly pine, however, was significantly reduced due to this higher concentration of benomyl. Pawuk and Barnett (1981) also reported the specific action of benomyl against antagonistic fungi to mycorrhizae to stimulate root association which benefited seedlings in their field performance.

Root growth potential of seedlings was reduced due to use of benomyl at 5 percent active ingredient in this experiment. This level has also destroyed roots of some of the treated seedlings.

Previous studies did not agree on the optimal quantity of fungicide used as a mixture of clay slurry for root treatment. Often concentrations recommended for controlling diseases were used which could be harmful to some species. While first-year performance of stored loblolly pine was adversely affected due to benomyl at 1.25 percent active ingredient in one study (Stumpff and South 1991), Barnett et al. (1988) reported no effect on survival of loblolly pine even at 5 percent active ingredient level. Barnett et al. (1988) have also found higher survival rates for late lifted loblolly and slash pine seedlings treated with benomyl at 1.25 percent active ingredient. Storage and field performance of longleaf and shortleaf pine seedlings were also improved by benomyl at 10 percent active ingredient (Barnett and Kais 1986) and 5 percent active ingredient (Kais and Barnett 1984, Barnett et al. 1988) respectively. Besides, Kais et al. (1986) concluded that a 5-percent active ingredient benomyl root treatment of longleaf pine seedlings prior to outplanting is safe and effective for field performance. They also indicated that soils with high silt content tend to retain fungitoxicant for a short period of time so that benomyl at 10 percent active ingredient was phytotoxic and reduced survival of seedlings. And it was suggested that lowering the concentration might have beneficial effect to seedlings as well as its effect on diseases. The improvement on RGP and field survival of shortleaf pine gained in this experiment was with treatment of benomyl at 0.5 to 1.0 percent active ingredient mixed in a clay slurry which were lower than most levels that were under investigation so far. All levels of benomyl above or below this range were either not effective or harmful to seedlings and their physiological activities.

Burdett (1987) pointed out that the capacity to produce new roots under laboratory condition correlates with the ability to perform better in the field. In the present experiment seedlings in controlled environment, growth chamber and greenhouse, have showed larger differences in RGP due to treatments than did seedlings in the field.

Throughout the experiment the activities of thiophanate-methyl on RGP or field performance were inconsistent. Therefore, further study is needed before thiophanate-methyl is recommended as an alternative to benomyl.

The lack of consistent significant interaction between clay and benomyl supports the idea that benomyl has at least some capacity to improve seedlings performance above and beyond its fungicide activity.

The effect of cytokinin on root growth is indirect through its direct effect on bud burst (Ritchie and

Dunlap 1980). Although previous investigations showed the possession of cytokinin-like activity by a number of benzimidazole derived fungicides, there was no discernible outcome from the preliminary investigation conducted on benomyl. But as to thiophanate-methyl, it was found out that, the lower the concentration the higher the tendency to increase radicle length of germinating seeds. At 5 parts per million (5 mg/l) the increase on radicle length was almost 30 percent over untreated seeds. This outcome is compatible with the report that indicated the need for higher dose of benomyl (effect of 5 mg/l benomyl equals 0.001 mg/l kinetin on soybean callus and 150 mg/l benomyl equals 5 mg/l kinetin on radish cotyledons) to act like cytokinin (Skene 1972), but was contrary to the report that stated the least effectiveness of thiophanate-methyl as a growth regulator among all benzimidazole fungicides (Thomas 1974).

Finally, provided that the activity of benomyl is unaffected by the surrounding environment of seedlings, results from this study signify the advantage of fungicide benomyl for better RGP and field performance of stored shortleaf pine seedlings if it is used at the right time with the level optimum to seedlings.

CHAPTER VI

CONCLUSIONS

1. Benomyl can improve RGP, field survival and growth when applied before storage regardless of whether clay slurry is applied.
2. Adding benomyl just before planting does not improve RGP.
3. The beneficial effect of benomyl on RGP is not due only to its control of pathogenic fungi encouraged by the application of clay slurry to seedlings before storage, but is also due to the stimulation of new roots.
4. Benomyl at 0.5 to 1.0 percent active ingredient is more effective at improving RGP than lower concentration and at 5.0 percent active ingredient benomyl has a negative effect.
5. Thiophanate-methyl has no beneficial effect on RGP or field performance.

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APPENDICES

APPENDIX A

TABLES

TABLE 1

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GROWTH CHAMBER, LIFTED FEBRUARY 1991 AND STORED FOR ONE MONTH

Source	DF	F Value	Pr > F
BLOCK	14	3.01	0.001
CLAY	2	0.21	0.807
BENOMYL	2	2.63	0.077
CLAY*BENOMYL	4	1.61	0.178
ERROR	112	MSE = 602.60	

TABLE 2

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GREENHOUSE, LIFTED FEBRUARY 1991 AND STORED FOR ONE MONTH

Source	DF	F Value	Pr > F
BLOCK	14	1.39	0.167
CLAY	2	0.59	0.555
BENOMYL	2	0.79	0.457
CLAY*BENOMYL	4	0.49	0.743
ERROR	112	MSE =1448.72	

TABLE 3

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GREENHOUSE, LIFTED NOVEMBER 1991 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	14	2.90	0.001
CLAY	2	1.24	0.292
BENOMYL	2	2.99	0.054
CLAY*BENOMYL	4	0.50	0.733
ERROR	112	MSE = 31.69	

TABLE 4

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GREENHOUSE, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	14	1.80	0.047
CLAY	2	3.76	0.026
BENOMYL	2	16.24	0.001
CLAY*BENOMYL	4	2.35	0.058
ERROR	112	MSE = 468.21	

TABLE 5

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON SURVIVAL OF SHORTLEAF PINE SEEDLINGS LIFTED NOVEMBER 1991 AND PLANTED IN THE FIELD AFTER ONE MONTH OF STORAGE

Source	DF	F value	Pr > F
REP	9	10.61	0.001
CLAY	2	1.71	0.187
BENOMYL	2	2.25	0.113
CLAY*BENOMYL	4	1.59	0.187
ERROR	72	MSE = 0.01	

TABLE 6

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON SURVIVAL OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	3.15	0.003
CLAY	2	0.03	0.968
BENOMYL	2	2.65	0.077
CLAY*BENOMYL	4	0.44	0.778
ERROR	72	MSE = 0.02	

TABLE 7

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON HEIGHT OF SHORTLEAF PINE SEEDLINGS LIFTED NOVEMBER 1991 AND PLANTED IN THE FIELD AFTER ONE MONTH OF STORAGE

Source	DF	F value	Pr > F
REP	9	32.79	0.001
CLAY	2	0.91	0.407
BENOMYL	2	3.15	0.049
CLAY*BENOMYL	4	1.31	0.275
ERROR	72	MSE = 23.03	

TABLE 8

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON HEIGHT OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	17.05	0.001
CLAY	2	0.75	0.478
BENOMYL	2	0.27	0.762
CLAY*BENOMYL	4	0.61	0.654
ERROR	72	MSE = 12.86	

TABLE 9

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON SURVIVAL VOLUME INDEX OF SHORTLEAF PINE SEEDLINGS LIFTED NOVEMBER 1991 AND PLANTED IN THE FIELD AFTER ONE MONTH OF STORAGE

Source	DF	F value	Pr > F
REP	9	11.71	0.001
CLAY	2	1.85	0.165
BENOMYL	2	2.99	0.057
CLAY*BENOMYL	4	0.97	0.429
ERROR	72	MSE = 4.34	

TABLE 10

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF CLAY AND BENOMYL ROOT TREATMENT ON SURVIVAL VOLUME INDEX OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	11.02	0.001
CLAY	2	1.09	0.343
BENOMYL	2	0.03	0.969
CLAY*BENOMYL	4	0.34	0.849
ERROR	72	MSE = 0.66	

TABLE 11

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF BENOMYL ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GROWTH CHAMBER, LIFTED FEBRUARY 1991 AND STORED FOR ONE MONTH

Source	DF	F Value	Pr > F
BLOCK	14	0.92	0.541
TREATMENT	4	7.23	0.001
ERROR	56	MSE = 616.06	

TABLE 12

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF BENOMYL ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GREENHOUSE, LIFTED FEBRUARY 1991 AND STORED FOR ONE MONTH

Source	DF	F Value	Pr > F
BLOCK	14	2.60	0.006
TREATMENT	4	15.78	0.001
ERROR	56	MSE = 1029.80	

TABLE 13

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GROWTH CHAMBER, LIFTED NOVEMBER 1991 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	14	2.76	0.002
TREATMENT	8	2.13	0.039
ERROR	112	MSE = 25.07	

TABLE 14

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON ROOT GROWTH POTENTIAL OF SHORTLEAF PINE SEEDLINGS IN GROWTH CHAMBER, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	14	1.85	0.040
TREATMENT	8	1.90	0.067
ERROR	112	MSE = 500.01	

TABLE 15

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON SURVIVAL OF SHORTLEAF PINE SEEDLINGS LIFTED NOVEMBER 1991 AND PLANTED IN THE FIELD AFTER ONE MONTH OF STORAGE

Source	DF	F value	Pr > F
REP	9	18.82	0.001
TREATMENT	8	1.37	0.227
ERROR	72	MSE = 0.01	

TABLE 16

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON SURVIVAL OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	1.90	0.066
TREATMENT	8	1.49	0.176
ERROR	72	MSE = 0.01	

TABLE 17

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON SURVIVAL VOLUME INDEX OF SHORTLEAF PINE SEEDLINGS LIFTED NOVEMBER 1991 AND PLANTED IN THE FIELD AFTER ONE MONTH OF STORAGE

Source	DF	F value	Pr > F
REP	9	11.87	0.001
TREATMENT	8	1.87	0.079
ERROR	72	MSE = 4.52	

TABLE 18

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON HEIGHT OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	21.71	0.001
TREATMENT	8	0.81	0.594
ERROR	72	MSE = 9.27	

TABLE 19

RESULTS OF THE ANALYSIS OF VARIANCE TO TEST FOR EFFECT OF RATE OF FUNGICIDES ON SURVIVAL VOLUME INDEX OF SHORTLEAF PINE SEEDLINGS PLANTED IN FIELD, LIFTED MARCH 1992 AND STORED FOR ONE MONTH

Source	DF	F value	Pr > F
REP	9	8.50	0.001
TREATMENT	8	0.93	0.498
ERROR	72	MSE = 0.40	

TABLE 20

RESULTS OF THE GENERAL LINEAR MODELS PROCEDURES TO LABORATORY TEST FOR EFFECT OF RATE OF FUNGICIDES ON GERMINATING SEEDS OF SHORTLEAF PINE

Source	DF	F value	Pr > F
BLOCK	3	0.09	0.966
TREATMENT	10	2.60	0.025
ERROR	26	MSE = 43.37	

APPENDIX B

FIGURE

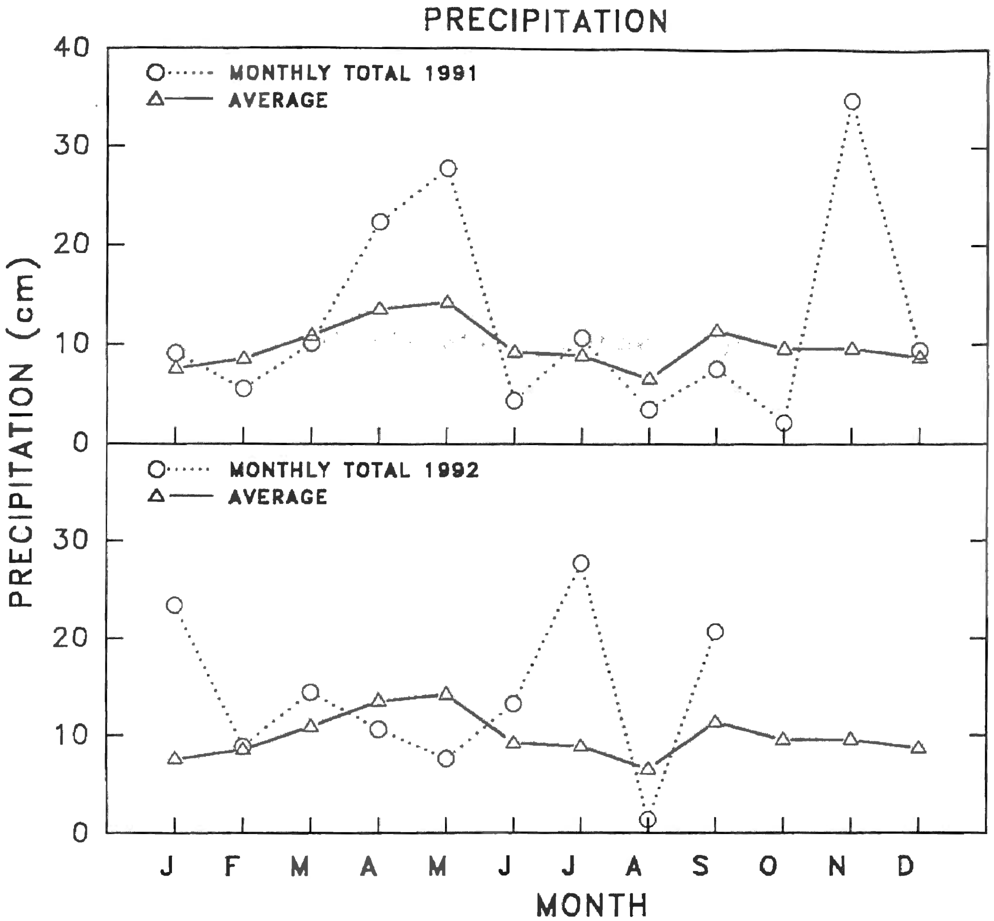

Figure 1. Monthly on-site precipitation for 1991 and 1992 and average monthly precipitation. Average monthly precipitation based on data recorded from 1951-1980 at Idabel

VITA 2

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Master of science

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