PRODUCTION PRACTICES OF ROSEMARY
(*ROSEMARINUS OFFICINALIS* L.) AND
SAGE (*SALVIA OFFICINALIS* L.)

by

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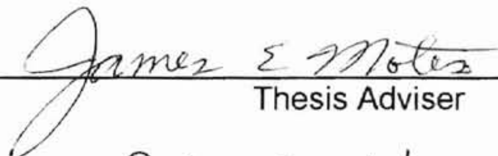
Thesis Approved:



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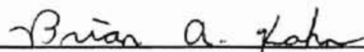

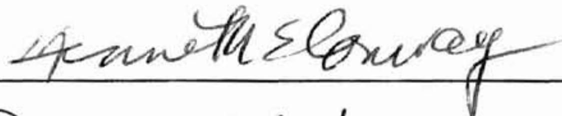

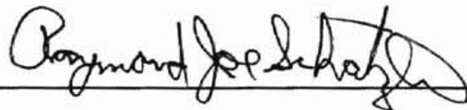

Dean of the Graduate College

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Overall Introduction

Herbs are valued for flavor, fragrance, or medicinal properties. Most herbs belong to three major families. Compositae includes artemesias, chamomile, tansy and yarrow. Umbelliferae includes caraway, coriander, dill, lovage and parsley. Labiatae, the mint family, includes basil, lavender, marjoram, the mints, rosemary, sage, and thyme (Clark, 1988).

Two herbs were involved in these studies, rosemary (Rosemarinus officinalis L. cultivar Arp) and sage (Salvia officinalis L. Dalmatian type). The primary objective for rosemary was to develop techniques to promote the propagation of healthy cuttings. There are various disease problems in rooting rosemary under a mist bench. Time of year was shown to be an important factor. The major disease causing agent was found to be Rhizoctonia solani (Conway et al., 1992). Use of biocontrol agents is very popular and was incorporated into this study. The overall objective for sage was to maximize yields.

Herbs are alternative or supplemental crops that offer an excellent diversification opportunity for farmers. This diversification may help stabilize a farm operation, increase farm income and benefit economic development. Much of the herb products used in the United States currently are imported from Africa,

Asia and Europe. Development of efficient domestic production should reduce imports and possibly increase exports which would have a small, but favorable, impact on the U.S. trade deficit. The U.S. is the world's largest importer of herbs. Limitation on domestic production predominately are economical, often related to the lack of grower familiarity with herb crops and the historical development and procurement of the raw products in exporting countries (Simons, 1987).

Many of the herbs in world commerce come from developing or third-world countries where hand labor is available and inexpensive. Herbs are often gathered from the wild by animal herders or foragers and collected by buyers that eventually sell to exporters of spices and herbs. Cultivated plots are generally very small family operations of a half acre or less.

These studies looked at mechanizing the harvest and handling of sage. The production and mechanized harvest is feasible according to Motes and Bostian (1990). Three aspects of herb culture in the field were addressed: plant population, date of last fall harvest, and nitrogen fertilizer requirements.

Plant population is important due to the relatively high cost of seedling transplants of sage. A lower plant population which yields an economically feasible production is the optimum scenario for the farm operation. These studies looked at four in-row plant spacings to find the optimal economic spacing.

The date of the last fall harvest is also an important aspect which these studies examined. The total number of harvests had been determined in earlier studies by Motes and Bostian (1990). For rosemary and sage, it was determined that late spring, summer, and fall were the necessary harvest times for optimum total yields allowing ample regrowth between harvests. It was necessary, however, to determine the effect of the last harvest in the fall, on long term plant health and future yields. Most literature recommended a September harvest, probably due to succulent growth.

Nitrogen fertilizer requirements are also very important in any plant's regime. Most literature recommends very low nitrogen be applied because of the effect on essential oils. Too much nitrogen causes a growth too fast and too lush for the essential oils to be very plentiful.

Rosemary is best planted in the field as a rooted cutting due to variability in plants produced from seed. Rosemary is very sensitive to soil pH, irrigation regime and heat in the first year of growth. Once established, 'Arp' rosemary is a hardy perennial in Zone 8 to Zone 10. The time of year to transplant is an important factor, both with rooting the cuttings and survival in the field. Preliminary work showed August was too late in the year to transplant the rooted cuttings because they will not survive the extreme summer temperatures and store enough root carbohydrates to survive the winter. DeBaggio (1990) stated that transplanting 'Arp' after July decreased the winter hardiness. Rosemary is

reported to tolerate temperatures as low as -15 °C to -23 °C (Hackett and Carolane, 1982).

Sage is best planted as a seedling because it would be too costly for rooted cuttings when such cuttings are not necessary. It is best grown in a well-drained, nitrogen rich clay loam. Ranges of soil pH vary and the reported tolerance is from 4.2 to 8.3. Sage is winter hardy down to -15 °C. Growth of sage requires full sunlight and low to moderate water. It is recommended that plantings should be replaced every three to four years because the plants become woody and quality decreases.

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CHAPTER I

IMPROVING PROPAGATION OF ROSEMARY (*ROSEMARINUS OFFICINALIS* L.) CUTTINGS UNDER A GREENHOUSE MIST SYSTEM

Abstract

Rosemary (*Rosemarinus officinalis* L. cultivar Arp) seed viability is low and seed that do germinate produce diverse plant types. Rosemary production requires asexual propagation to insure uniformity in the field. Hormone application methods were compared using greenhouse and field collected cuttings. No significant differences existed due to source of cuttings. Five concentrations of indole butyric acid (IBA) and three methods of application were used in this study. Results indicated a 2 min soak in captan, benomyl, and streptomycin solution followed by dipping the cutting into 0.8% IBA/talc consistently resulted in higher quality rooted cuttings than other treatment combinations.

Introduction

Rosemary is an evergreen perennial shrub native to the western
diterranean region (Foster, 1986). In addition to its traditional
ization for essential oils, rosemary is being increasingly utilized for its
ioxidant properties. Antioxidants are used in human and animal
ds as a preservative. According to research by Chipault *et al.* (1952)
32 spices studied, rosemary and sage (*Salvia officinalis*) were reported
be particularly effective as sources of antioxidants. Also, Bracco *et al.*
)81) showed how molecular distillation of rosemary derivatives could
)tect foods against oxidative rancidity. The antioxidant rosmariquinone
is isolated and identified by Houlihan *et al.* (1985) from the leaves of
semarinus officinalis L. Industry is continually searching for natural
tioxidants, which are more appealing to the health conscious world, and
search is ongoing. Currently, most antioxidants are manufactured
ough synthetic chemical processes. However, there is increasing
nsumer pressure for the use of "natural" preservatives in foods which
ovides a demand for the antioxidants which can be extracted from
semary.

In order to meet the need for large scale propagation of rosemary a
imber of problems need to be solved. Seed viability is low and seed
at do germinate produce a diverse group of plant types (DeBaggio,
)90). Direct seeding is uncommon in commercial fields. To keep
iiformity and high plant quality in rosemary, asexual propagation is

necessary.

The objective of this study was to develop a quick method of rooting rosemary cuttings on a large scale. Rosemary can be relatively difficult to propagate. The literature states survival of rooted cuttings is decreased by two major problems, various root rot pathogens and powdery mildew (*Podosphaera leucotricha*) (Kowalchik and Hylton, 1987). Other studies identified *Rhizoctonia solani* as the major cause of aerial blight and root rot of cuttings during mist propagation (Conway *et al.,* 1992). It would be less costly to propagate rosemary on a mist bench if a quick method for rooting was developed that had fewer disease problems. Quickness of rooting is dependent on the health of the stock plant and the time of the year (Kowalchik and Hylton, 1987). Hormonal treatment is recommended to stimulate root growth; otherwise, rosemary cuttings may take several months to root (Clark, 1988). However, the optimum concentration of hormone is unclear. This experiment was designed to determine the concentration of hormone that would most effectively induce rooting and determine the optimum method of application.

Materials and Methods

Four concentrations of indole butyric acid (IBA) were used (0.8, 1.6, 3.0 and 4.5 percent IBA) in talc powder in addition to the control. Three methods of applying IBA were used: a bath/dip, soil drench, and a 2 min bath. Rosemary cuttings were 13 cm long and the lower 5 cm was

In order to use the biocontrol fungi for integration with fungicides, isolates of *Trichoderma harzianum* Rifai (OK-110) and *Laetisaria arvalis* Burdsall (OK-206) were plated onto agar amended with a ten-fold dilution series of the fungicides: Rovral (iprodione) and CGA 173506, respectively, and when growth of the colony was observed, hyphal tip transfers were made to non-amended agar. After 7-days growth, the colony was again transferred back to amended agar to verify that reversion had not occurred. Biological control agents and rates used were derived from previous research (4,5,6,7). Isolates of both biological agents have effectively control *R. solani* and *Sclerotium rolfsii* Sacc. in field soil (4,5).

Fungicide treatment

Fungicides and rates evaluated were CGA-173506 (Ciba-Geigy Corp.) (0.07 g /473 ml) (approximately ½ x label rate) and Rovral (RhonePoulenc Inc.)(iprodione)(1.2 ml /473 ml) (Label rate). Controls received no fungicide application, soil amendment or pathogen inoculation.

Nuclear condition and hyphal anastomosis

The number of nuclei per cell of *R. solani* isolate (OK-367) was determined with flourescence microscopy and acridine orange stain. The anastomosis group of isolate OK-367 was determined on agar-coated slides (11), with cultures of Prof. Ogoshi obtained from R. J. Cook (20).

in the evening after the mist was off. After seven to 10 d, when most of the cuttings had roots initiated, a fertilizer drench was applied using 0.75g·L of 15N-13.2P-12.4K.

Speedling styrofoam-100A flats (Speedling, Inc., P.O. Box 129, Sun City, FL, 34028) were used to root the cuttings. There were 20 rows in each flat and every other row was filled with medium. The two outside rows were guards. The eight rows in the middle received designated treatments. Each row included 10 cuttings. Each replication consisted of three flats which were kept together in the daily rotation of the flats on the bench. There were eight replications with 10 cuttings per replication. This study was done in three consecutive years.The statistical design was a randomized complete block and analyzed accordingly.

Weekly shoot ratings were made, counting the number of diseased or "browning" cuttings. These data were used for another experiment as an indicator of the quickness of disease infestation across the greenhouse flat. The disease organism was identified as *Rhizoctonia solani* AG-4 and control was pursued (Conway *et al.,* 1992). This experiment is explained in detail in Chapter II.

Three weeks after hormonal treatment, the cuttings were pulled and the medium was washed away from the roots. Roots were rated on a scale of 1 to 5, with '1' showing no roots initiated and '5' showing a full mat of roots along the entire 4 cm that had been inserted into the medium. Roots were removed, dried, and weighed. The experiments were

scheduled for late fall of each year because the time of year greatly affects the rate of rooting in rosemary (Kowalchik and Hylton, 1987). Also, preliminary experiments had shown disease incidence to be significantly higher in the spring or summer, as compared to the fall.

Results and Discussion

There was no significant difference between the greenhouse collected cuttings which were of soft wood and the field collected cuttings which were of hard wood.

Root ratings are illustrated in Figure 1. Results of these ratings are shown in Figure 2 for 1989, Figure 3 for 1990, and Figure 4 for 1991. In 1989, there were no significant differences between IBA concentrations and method of application as shown in Figure 2. However, the numerically highest rating occurred using 0.8% IBA applied as a bath/dip. In 1990, the 0.8% IBA applied as a bath/dip resulted in the significantly highest root ratings (Figure 3). The 1.6% IBA applied as a drench or applied as a bath/dip resulted in the highest root ratings in 1991 (Figure 4). Also, there was a tendency for a low root rating with 4.5% IBA when applied as a bath/dip as compared to the other two methods of application in 1991. This was also seen in 1989 and is due to an IBA concentration being too high for rosemary when applied directly to the cutting. The shift in effectiveness among IBA strengths from the first two years to the third year is due to climatic conditions. In 1989 and 1990, the

experiments were conducted in October. The 1991 study was conducted in January and only with greenhouse collected cuttings since field plants were semi-dormant.

Actual root dry mass is shown in Figures 5 and 6 for 1989, Figures 7 and 8 for 1990 and Figures 9 and 10 for 1991. Actual root mass and root ratings along with standard deviations are listed in Tables 1, 2 and 3 for 1989, 1990 and 1991, respectively.

In Figures 5, 7, and 9, it can be seen that the bath/dip method of application is the optimum method resulting in the highest root dry mass in all three years irrespective of the climatic conditions outside the greenhouse. In the October experiments of 1989 and 1990, 0.8% and 1.6% IBA gave the greatest root mass. In the January experiment of 1991, 1.6% and 3.2% IBA gave the greatest root mass. These conclusions are also apparent in Figures 6, 8, and 10 where the root mass are charted by IBA concentrations.

One of the purposes of this study was to determine if there is the possibility of a more automated system of applying hormone and disease control methods in a rosemary rooting regime. These results show a hormone/talc dip is still necessary to ensure optimum rooting. If a drench or soak is desirable, a higher hormone concentration is needed depending on climatic conditions. However, insufficient data was collected to determine if disease control measures could also be implemented in an automated system.

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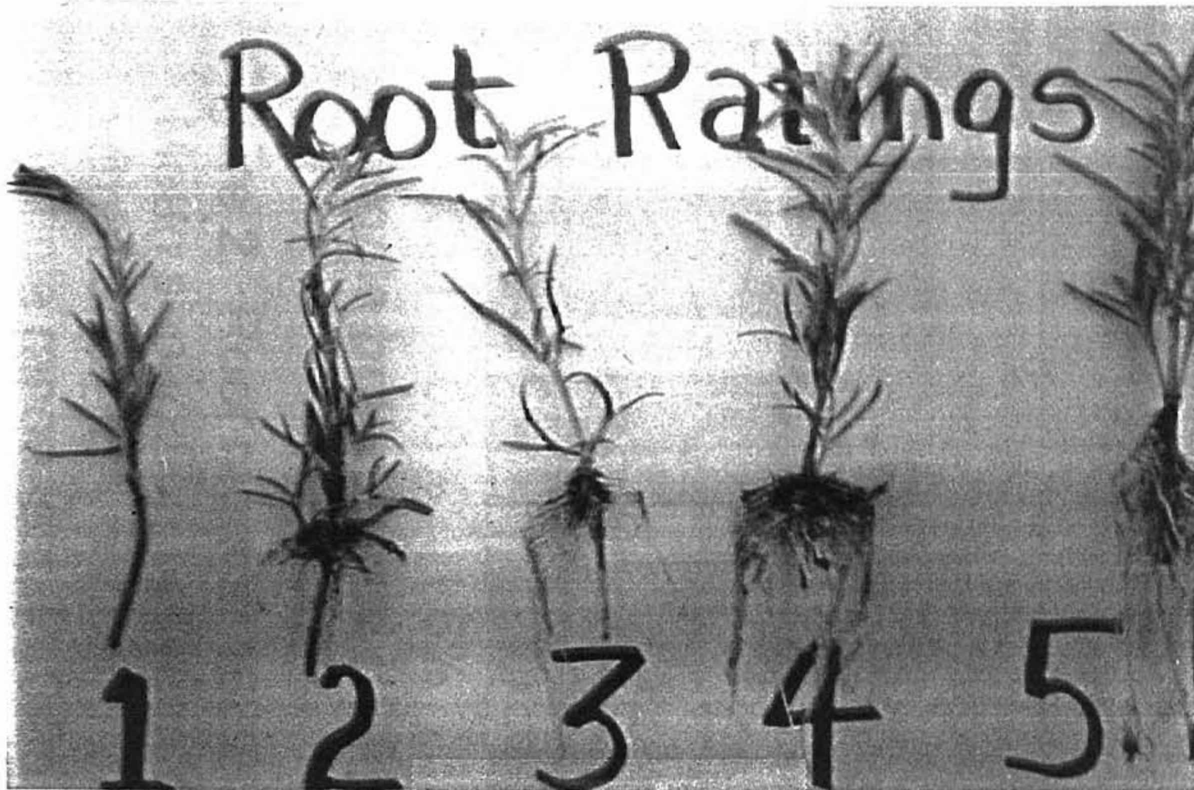

Figure 1. Root ratings in rosemary, 1=poor, 5=excellent.

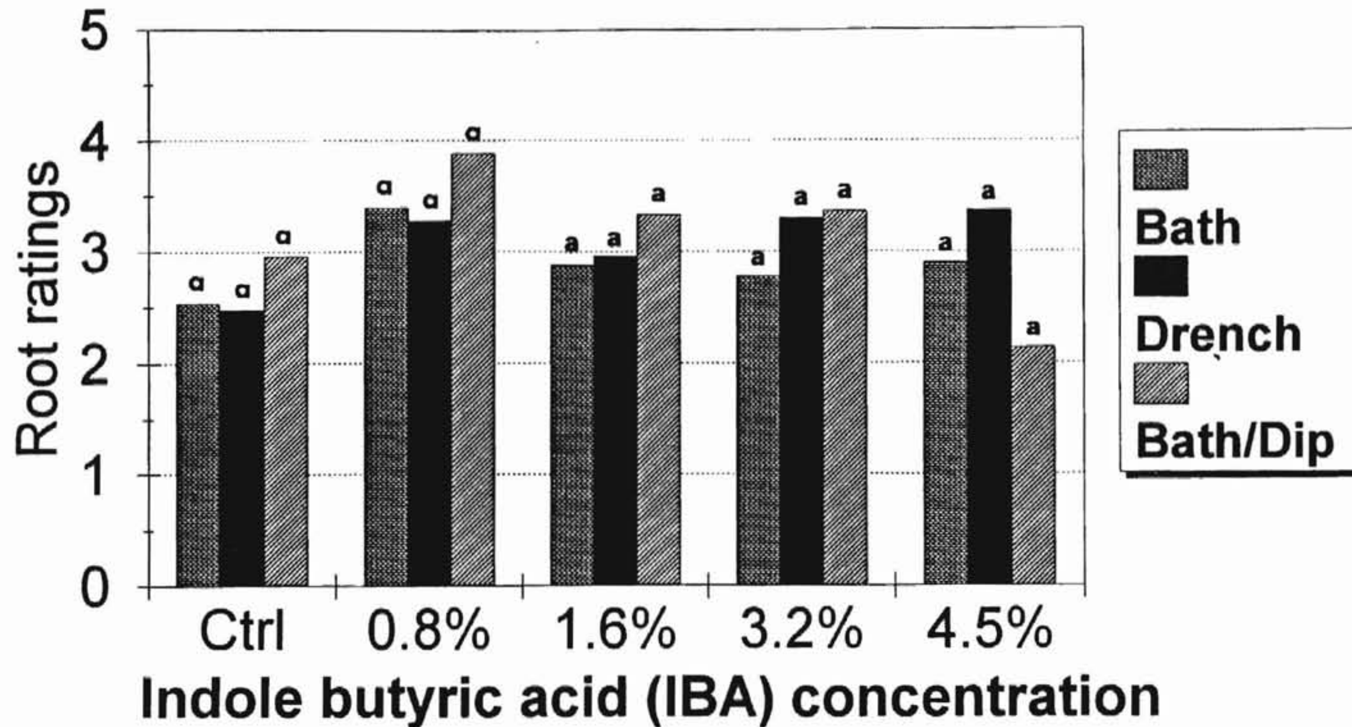

Figure 2. Root ratings, 1989, rosemary rooting study. Mean separation between method of application within each IBA concentration by Duncan's new multiple range test, $P \leq 0.05$.

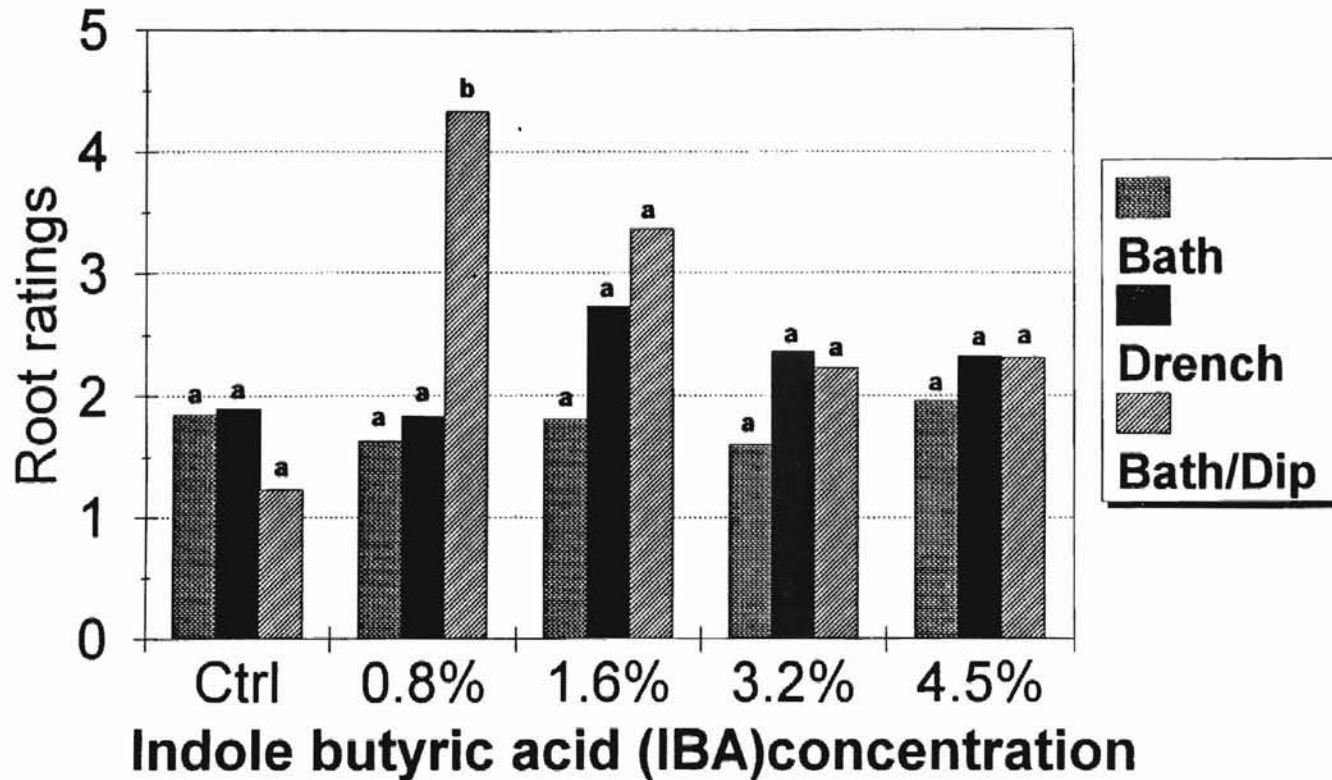

Figure 3. Root ratings, 1990, rosemary rooting study. Mean separation between method of application within each IBA concentration by Duncan's new multiple range test, $P \leq 0.05$

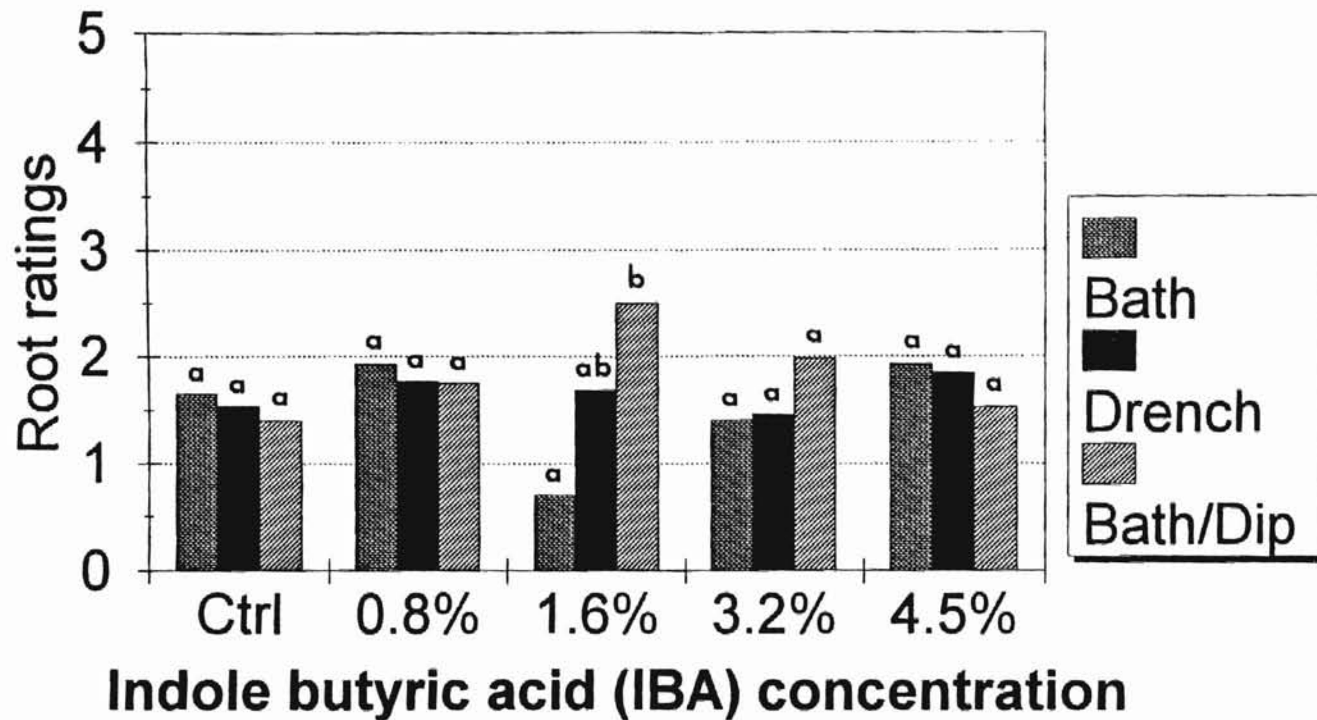

Figure 4. Root ratings, 1991, rosemary rooting study. Mean separation between method of application within each IBA concentration by Duncan's new multiple range test, P≤0.05.

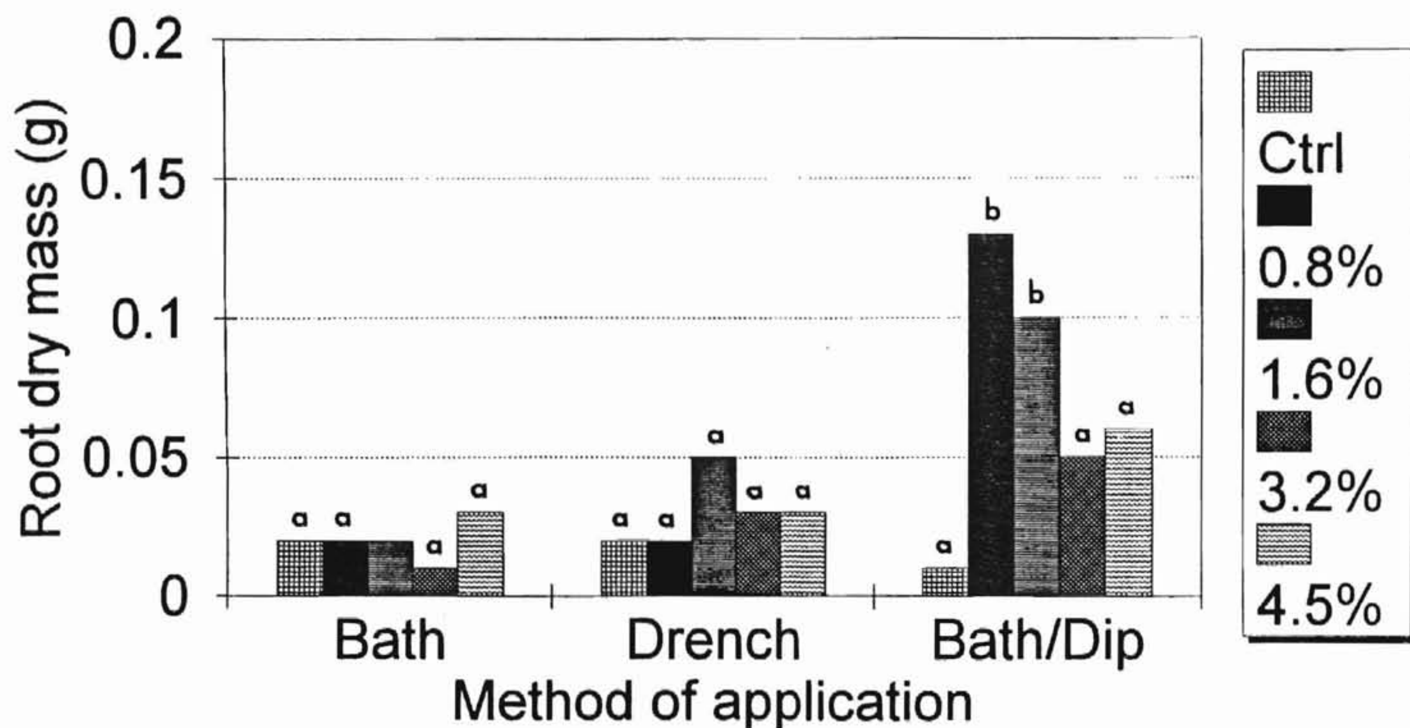

Figure 5. Root dry mass (g) compared by method of application in rosemary rooting study, 1989. Mean separation between IBA concentrations within each method of application by Duncan's new multiple range test, $P \leq 0.05$.

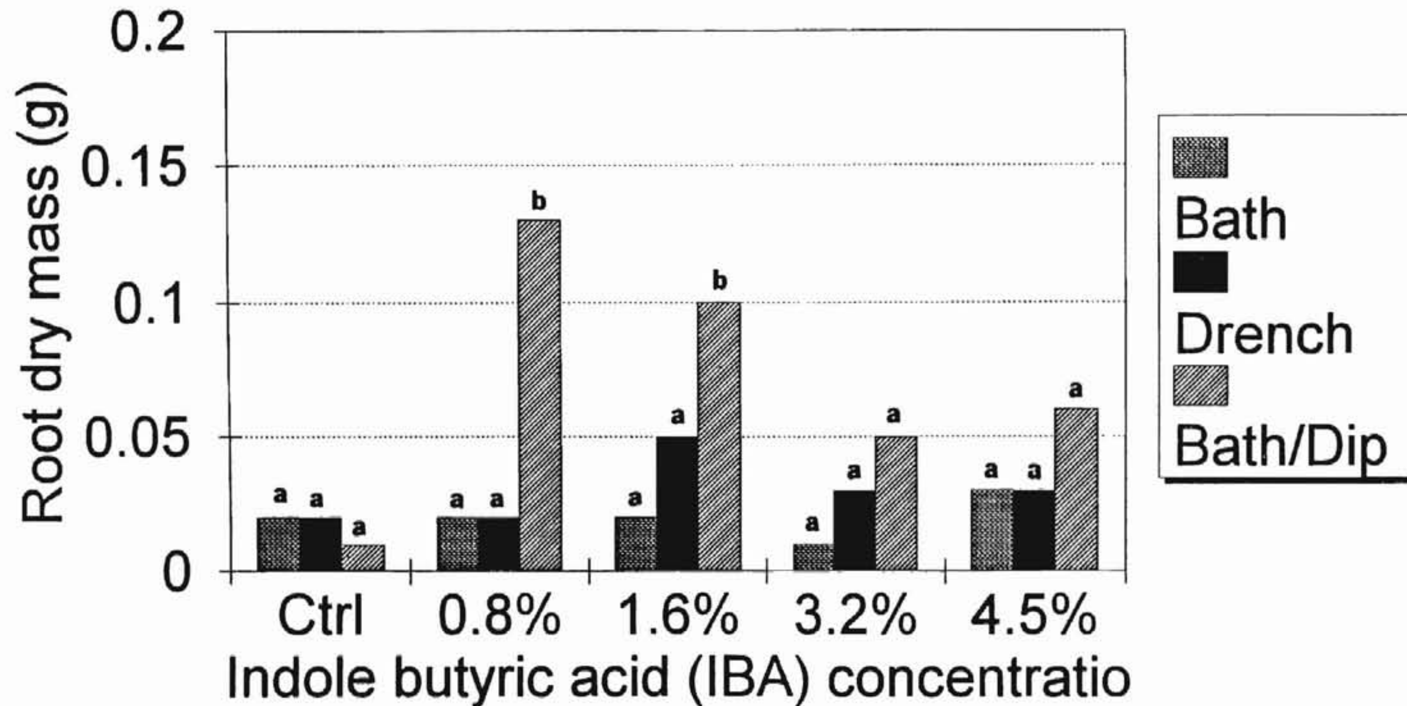

Figure 6. Root dry mass (g) by indole butyric acid (IBA) concentration in rosemary rooting study, 1989. Mean separation between method of application within IBA concentrations by Duncan's new multiple range test, $P \leqslant 0.05$.

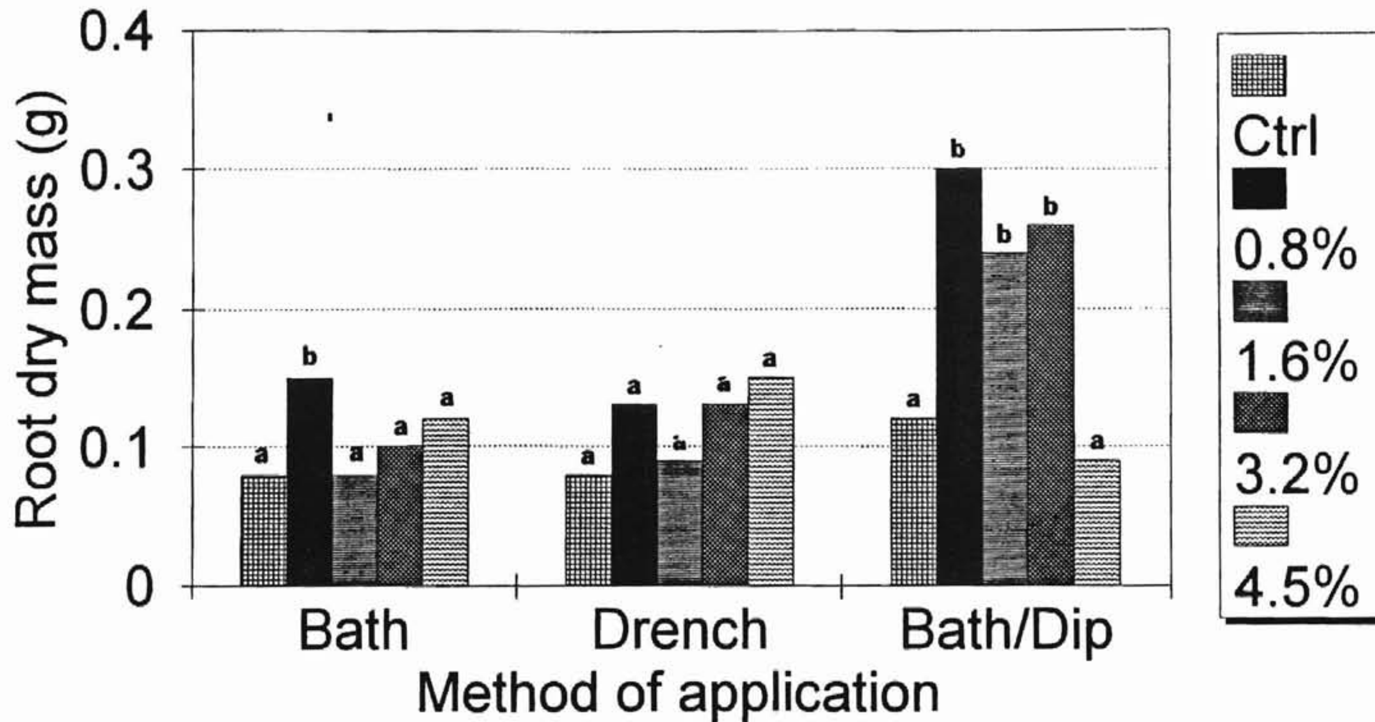

Figure 7. Root dry mass (g) compared by method of application in rosemary rooting study, 1990. Mean separation between IBA concentrations within method of application by Duncan's new multiple range test, $P \leqslant 0.05$.

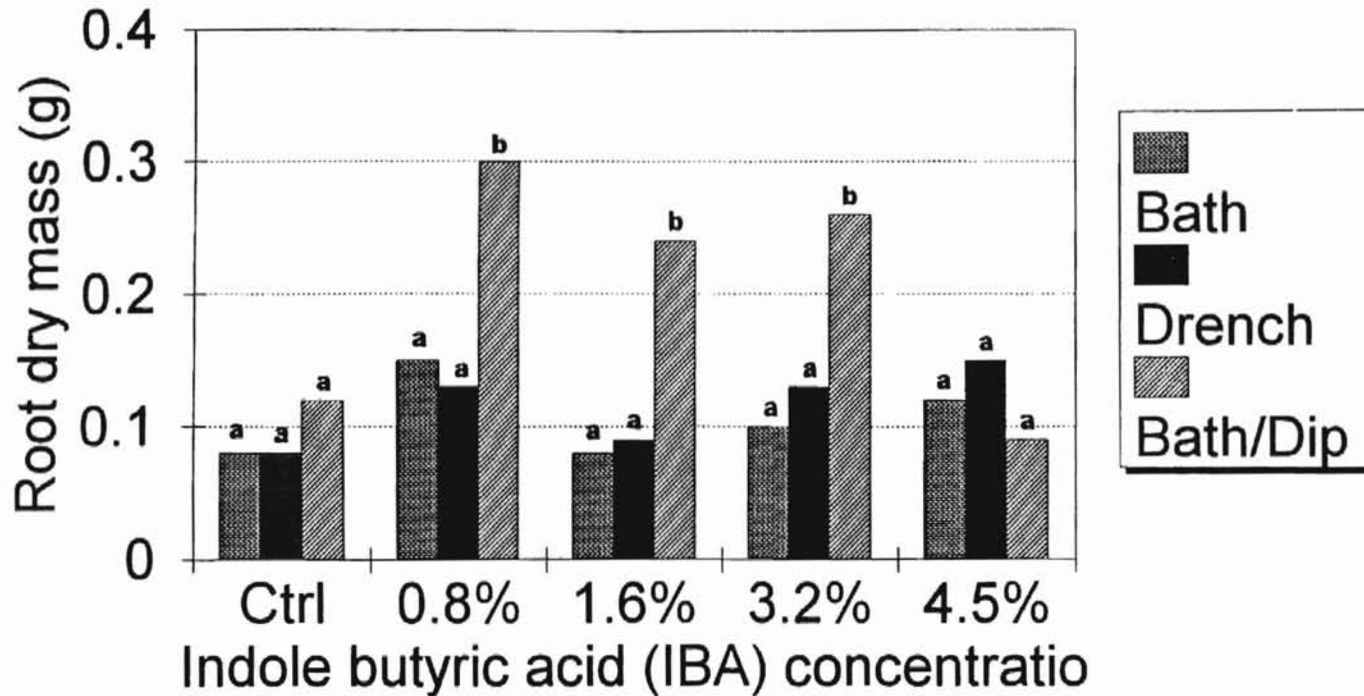

Figure 8. Root dry mass (g) compared by indole butyric acid (IBA) concentration in rosemary rooting study, 1990. Mean separation between method of application within each IBA concentration by Duncan's new multiple range test, P 0.05.

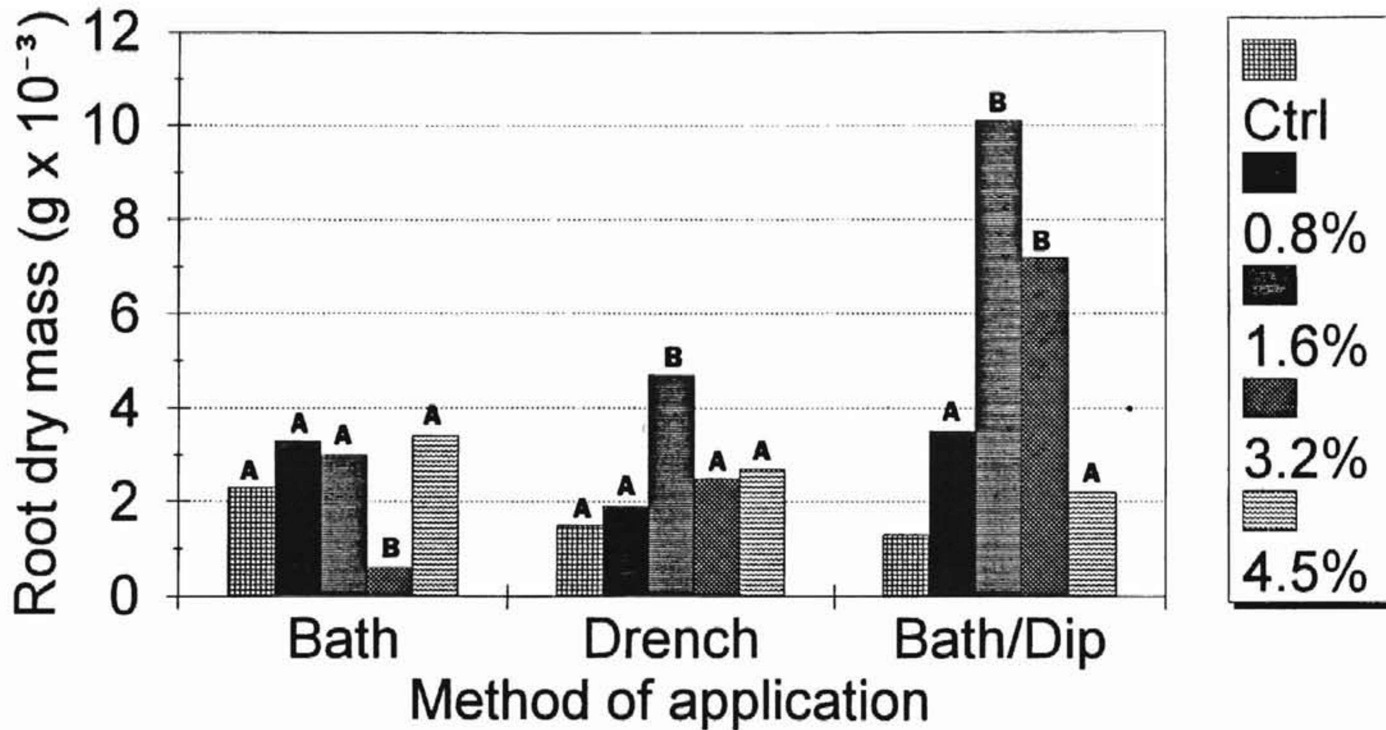

Figure 9. Root dry mass, 1991, rosemary rooting study, 1991. Mean separation between IBA concentrations within method of application by Duncan's multiple range test, P⩽0.05.

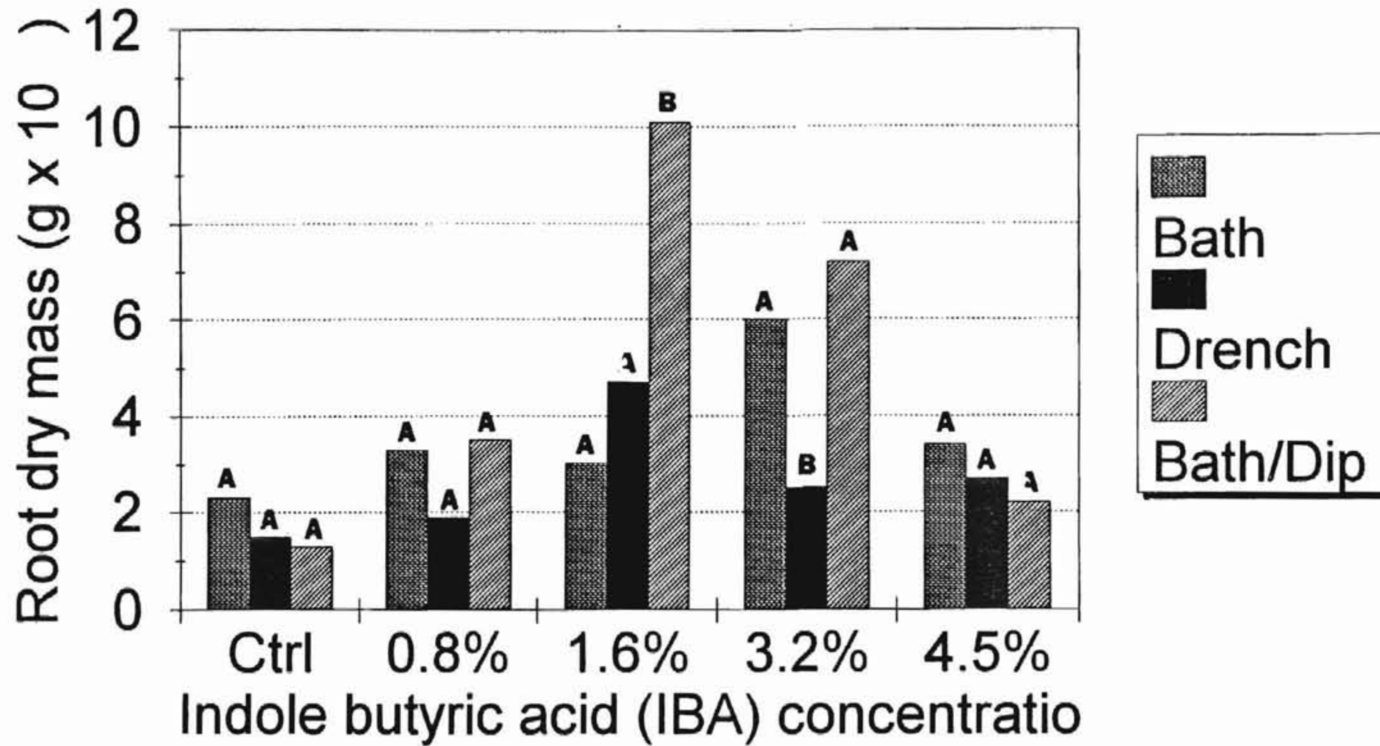

Figure 10. Root dry mass, 1991, rosemary rooting study. Mean separation between method of application within IBA concentrations by Duncan's new multiple range test, $P \leq 0.05$.

Table 1. Root mass and root ratings with standard deviations for rosemary cuttings treated with five hormone concentrations and three methods of application in 1989.

Method of application	Hormone concentration	Root mass (mg)	Std. dev.	Root rating[z]	Std. dev.
Bath[y]	Control	17	24	1.9	0.8
Drench[x]	Control	20	23	1.9	0.9
Bath/Dip[w]	Control	3	7	1.2	0.4
Bath	0.8%[v]	17	26	1.6	0.8
Drench	0.8%	17	25	1.8	1.0
Bath/Dip	0.8%	130	49	4.3	0.9
Bath	1.6%	19	27	1.8	0.9
Drench	1.6%	48	42	2.7	1.3
Bath/Dip	1.6%	103	81	3.4	1.6
Bath	3.2%	12	21	1.6	0.9
Drench	3.2%	35	32	2.4	1.1
Bath/Dip	3.2%	46	59	2.2	1.4
Bath	4.5%	25	31	2.0	0.9
Drench	4.5%	31	36	2.3	1.2
Bath/Dip	4.5%	62	63	2.3	1.2

[z]Root ratings based on 1=no roots, and 5=full mat of roots along entire 4 cm base of the cutting which was inserted in the medium.

[y]Bath consisted of 6 g captan, 3 g benomyl, ½ g streptomycin and 2 g of the respective IBA/talc mixed into 3.85 L of water; then agitated for 2 min with cuttings immersed.

[x]Drench consisted of the same as bath but was applied with a dropper to the base of the cutting after being inserted in the medium.

[w]Bath/dip consisted of the same ingredients minus the IBA/talc. After the 2 min agitated bath, cuttings were dipped into respective IBA/talc, then inserted in the medium.

[v]Concentration of indole butyric acid/talc.

Table 2. Root mass and root ratings with standard deviations for rosemary cuttings treated with five hormone concentrations and three methods of application in 1990.

Method of application	Hormone concentration	Root mass (mg)	Std. dev.	Root rating[z]	Std. dev.
Bath[y]	Control	8	7	2.3	0.06
Drench[x]	Control	8	7	2.7	0.09
Bath/Dip[w]	Control	12	10	3.2	0.17
Bath	0.8%[v]	15	10	3.1	0.11
Drench	0.8%	13	9	3.0	0.09
Bath/Dip	0.8%	30	20	3.8	0.08
Bath	1.6%	8	5	3.2	0.10
Drench	1.6%	9	7	2.2	0.09
Bath/Dip	1.6%	24	18	3.0	0.14
Bath	3.2%	10	10	2.9	0.15
Drench	3.2%	13	7	3.2	0.08
Bath/Dip	3.2%	26	14	3.6	0.09
Bath	4.5%	12	7	2.7	0.09
Drench	4.5%	15	8	3.5	0.08
Bath/Dip	4.5%	9	9	1.7	0.12

[z]Root ratings based on 1=no roots, and 5=full mat of roots along entire 4 cm base of the cutting which was inserted in the medium.

[y]Bath consisted of 6 g captan, 3 g benomyl, ½ g streptomycin and 2 g of the respective IBA/talc mixed into 3.85 L of water; then agitated for 2 min with cuttings immersed.

[x]Drench consisted of the same as bath but was applied with a dropper to the base of the cutting after being inserted in the medium.

[w]Bath/dip consisted of the same ingredients minus the IBA/talc. After the 2 minute agitated bath, cuttings were dipped into respective IBA/talc, then inserted in the medium.

[v]Concentration of indole butyric acid/talc.

Table 3. Root dry mass and root ratings with standard deviations of each for rosemary cuttings treated with five hormone concentrations and three methods of application, in 1991.

Method of application	Hormone concentration	Root mass (mg)	Std. dev.	Root rating[z]	Std. dev.
Bath[y]	Control	2	5	1.7	1.0
Drench[x]	Control	2	4	1.5	0.9
Bath/Dip[w]	Control	1	5	1.4	0.9
Bath	0.8%[v]	3	6	1.9	1.1
Drench	0.8%	2	3	1.8	0.8
Bath/Dip	0.8%	4	8	1.8	1.2
Bath	1.6%	3	8	1.7	1.0
Drench	1.6%	5	10	1.7	1.1
Bath/Dip	1.6%	10	17	2.5	1.4
Bath	3.2%	1	2	1.4	0.7
Drench	3.2%	3	7	1.5	0.9
Bath/Dip	3.2%	7	11	2.0	1.3
Bath	4.5%	3	1	1.9	1.1
Drench	4.5%	3	5	1.9	1.1
Bath/Dip	4.5%	2	6	1.5	0.9

[z]Root ratings based on 1=no roots, and 5=full mat of roots along entire 4 cm base of the cutting which was inserted in the medium.

[y]Bath consisted of 6 g captan, 3 g benomyl, ½ g streptomycin and 2 g of the respective IBA/talc mixed into 3.85 L of water; then agitated for 2 min with cuttings immersed.

[x]Drench consisted of the same as bath but was applied with a dropper to the base of the cutting after being inserted in the medium.

[w]Bath/dip consisted of the same ingredients minus the IBA/talc. After the 2 min agitated bath, cuttings were dipped into respective IBA/talc, then inserted in the medium.

[v]Concentration of indole butyric acid/talc.

CHAPTER II

INTEGRATION OF BIOLOGICAL AND CHEMICAL CONTROLS FOR *RHIZOCTONIA* AERIAL BLIGHT AND ROOT ROT OF ROSEMARY

ABSTRACT

Aerial blight, caused by *Rhizoctonia solani* AG-4, was identified as a major disease of greenhouse mist-produced rosemary cuttings. An isolate of the biocontrol fungus *Laetisaria arvalis*, selected for tolerance to the experimental fungicide CGA 173506, was used as an amendment to potting soil. When combined with a foliar-spray of the fungicide (½ x label rate), it reduced disease greater than either treatment used alone. Synergism was not observed for combinations of *Trichoderma harzianum* soil amendment and a foliar-spray (applied at the label rate) of the fungicide iprodione (Rovral).

Introduction

Rosemary (*Rosemarinus officinalis L.*) grown from seed produce a wide variety of plant types, and seed viability is low (8). To maintain uniformity among rosemary plants, asexual propagation is necessary; however, rosemary is difficult to propagate (12). Generally, there are two major problems with rosemary cuttings: root rot and powdery mildew (10). *Rhizoctonia solani* Kjhn, a soilborne fungus, drastically decreases the survival rate of rooted cuttings (6). *R. solani* survives in infected roots which can become embedded in the side walls of Speedling styrofoam-100A flats (Speedling, Inc., P.O. Box 129, Sun City, FL, 34028) commonly used for propagation. These infected roots are resistant to sterilization procedures, such as Chlorox washes, and can reinfest the potting mixes during propagation. Spread of the pathogen is extremely rapid once the root and main stem are infected. The fungus moves upward along the stem, causing an aerial blight in which the fungus can rapidly infect an entire flat in just a few days by ectotrophically growing from plant to plant.

Our control strategy was to add a biological control agent to the potting mix to control infestation by *R. solani* by attacking the pathogen before it could infect the rosemary root. Foliar application of the fungicide would delay aerial blight development, but would not interfere with the biological control agent due to the selected tolerance. We believed that an integrated system could improve the reliability of biological control systems and could be synergistic (17). Preliminary reports of this research have been presented

in abstract form (6,18,19). This paper presents results of the inoculum density-disease relationship between our formulation of *R. solani* and rosemary cuttings, the effect of inoculum placement on disease development, the selection of biocontrol fungi for tolerance to selected fungicides and their ability to control *R. solani* aerial blight and root rot when used individually or in conjunction with fungicides.

Materials and Methods

Rosemary propagation

Cuttings taken from greenhouse grown plants are usually 9-18 cm long with the lower half of the foliage removed (3). Cuttings from each source plant were assayed on a modified *Rhizoctonia*-selective medium (9). Each liter of modified medium contained; agar, 20 g, inulin, 5 g, benomyl, 6 mg (a.i), copper sulfate, 21 mg, chlorotetracycline HCL, 70 mg. Only pathogen-free plants were used as source plants for our experiments. Previous research projects showed that 0.08% indole butyric acid powder/talc dip gave the best rooting (19). Cuttings were rooted on mist benches in a greenhouse in May and July, 1992. Fafard potting soil mix (Conrad Fafard, Inc., P.O. Box 7790, Aquawam, MA, 01001) was placed into plastic "six-pack" rooting containers, one hormone-treated rosemary seedling was placed into each cell of the six-pack.

Biocontrol treatments

In order to use the biocontrol fungi for integration with fungicides, isolates of *Trichoderma harzianum* Rifai (OK-110) and *Laetisaria arvalis* Burdsall (OK-206) were plated onto agar amended with a ten-fold dilution series of the fungicides: Rovral (iprodione) and CGA 173506, respectively, and when growth of the colony was observed, hyphal tip transfers were made to non-amended agar. After 7-days growth, the colony was again transferred back to amended agar to verify that reversion had not occurred. Biological control agents and rates used were derived from previous research (4,5,6,7). Isolates of both biological agents have effectively control *R. solani* and *Sclerotium rolfsii* Sacc. in field soil (4,5).

Fungicide treatment

Fungicides and rates evaluated were CGA-173506 (Ciba-Geigy Corp.) (0.07 g /473 ml) (approximately ½ x label rate) and Rovral (RhonePoulenc Inc.)(iprodione)(1.2 ml /473 ml) (Label rate). Controls received no fungicide application, soil amendment or pathogen inoculation.

Nuclear condition and hyphal anastomosis

The number of nuclei per cell of *R. solani* isolate (OK-367) was determined with flourescence microscopy and acridine orange stain. The anastomosis group of isolate OK-367 was determined on agar-coated slides (11), with cultures of Prof. Ogoshi obtained from R. J. Cook (20).

Inoculum production

Inoculum of *R. solani* was prepared by a method developed by M. G. Boosalis (personal communication). A 33 X 23 cm cake pan was partially filled with vermiculite, covered with aluminum foil and autoclaved (121°C, 1.05 kg/ cm2, 15 min). In a separate container, 400 to 500 ml of corn meal was covered and autoclaved three times. The autoclaved corn meal and vermiculite was mixed in the cake pan, and 550 ml sterile tap water was added. Agar and mycelium in 3 to 4 petri dishes of actively growing *R. solani* was cut into cubes and added to each pan, mixed thoroughly, and incubated 2 to 3 weeks at room temperature. The mixture was loosened from the pans, spread on a tray, covered with cheesecloth and allowed to dry overnight at room temperature. The mixture was further separated using a rolling pin. For sclerotial development, wheat bran was substituted for cornmeal. The mixture was placed in paper bags and stored at room temperature. For further refinement, the mixture was sieved through nested 500 and 250 mm screens for greater particle uniformity.

Inoculum Density-Disease Incidence (ID-DI) Relationship

Inoculum was prepared and mixed into the growing media with a twin-shell blender (Patterson-Kelly Co., East Stroudsberg, PA,18301) on a percentage by weight basis: 0, 0.01, 0.1, and 1.0%. Biocontrol agents were added into the blender to achieve desired propagule densities. ID-DI

Experiments were arranged in Latin-squares on greenhouse benches. Cuttings were evaluated weekly for disease incidence; brown discoloration and/or lesions, on the leaves. At four weeks, cuttings were removed and roots were evaluated for discoloration, dried and weighed. Each treatment was replicated 8 times. Numbers of dead plants in each treatment were subjected to Analysis of Variance and means separated by a Student-Newman-Keuls mean separation test. The experiment was conducted twice in a greenhouse, in May and June 1992.

Disease control studies: inoculum methods

Two methods for pathogen inoculation were evaluated to simulate both aerial and soil spread of the disease. Controls received no fungicide application, soil amendment, or pathogen inoculation. Fafard potting soil mix was placed into plastic "six-pack" rooting containers and one hormone-treated rosemary seedling was placed into each planting-cell. Inoculation of *R. solani* was accomplished using two different techniques: in the first, to simulate aerial blight plants in individual six-packs were misted with water and dusted with 0.1 g of the *R. solani* mix, and in the second method, to simulate the soil spread of the pathogen a 1.0 cm diameter disc removed from an actively growing culture of *R. solani* was placed on the potting soil of one of the end cells of the "six-pack". Fungicides were applied to run-off either before (protectant spray) or after (therapeutic spray) inoculation of the pathogen. Treatments were replicated eight times and were randomly

arranged in a complete block design inside a mist chamber. Numbers of dead plants were recorded daily for 2 weeks. Surviving plants were removed and primary roots were measured, and secondary roots trimmed from the primary root, and weighed (fresh weight). An ANOVA was conducted on the numbers of dead plants and the length and weight of the roots. When significance was indicated, means were separated using a Student-Newman-Keuls test (P=0.05). Each experiment was performed at least two times.

Disease Control Studies: Integration

Both fungi were mixed with Fafard potting soil mix. *T. harzianum* was prepared as a molasses-bran-fermentation product (13) in a Hi-Density Lab-Line Fermentor System (No. 29500) (LabLine Instruments, Inc., Melrose Park, IL). After one-week of growth, Mycelial fragments and conidia were collected on a 500 mm sieve, spread on wax paper, dried and ground to a fine powder in a Glen Mills grinder (Glen Mills Co., Maywood, NJ). *T. harzianum* was added at the rate of (5.0 g fermentation product /kg or 106 cfu /g of potting soil). *L. arvalis* was grown on Potato dextrose broth and sclerotia were separated from the mycelium by washing through nested sieves (500 mm, 250 mm and 180 mm). Sclerotia were collected on the 250 mm screen and placed on waxpaper to dry. *L. arvalis* was added to the potting soil mix as 5.0 g dried sclerotia /kg. Moisture content of the potting soil was adjusted to 20% (w/v) with water prior to the addition of biocontrol agents or pathogen. Biocontrol fungi were used alone and in combination

with the fungicides. Rosemary cuttings were stuck into individual cells of a Speedling 100-A propagation tray (100 rooting cells per tray). Trays were cleaned and disinfested with Chlorox and coated with Speedling Super-Cote (a copper-based latex paint) to inhibit *R. solani* infection from mycelium embedded in the styrofoam of the tray. Inoculum was incorporated into the potting soil on a percentage by weight basis prior to the addition of the biocontrol agents. Each tray contained seven treatments in every-other row. Skips were left between treatment rows to inhibit the spread of *Rhizoctonia* between treatments and to allow more accurate application of treatments. Fungicides were sprayed until run-off. Each treatment was replicated four times and there were 10 plants per rep. All trays were placed inside a misting unit for 19 days and the numbers of dead plants were recorded on a daily basis. Controls were planted into non-amended potting soil and received no fungicide.

Results and Discussion

Our isolate OK-367 of *R. solani* was multinucleate and fused with only the AG-4 tester culture of Ogoshi (18). The addition of 0.1% of the Rhizoctonia mix to Fafard potting soil produced approximately 50% disease (Fig. 1). The development of disease recorded for the control treatment (Fig. 1) illustrates how easily disease spreads from plant to plant during aerial blight. Higher disease incidence was noted during periods of time when warmer temperatures were present in the greenhouse (July vs May: May, daily

high temp: 24.5°C, low temp: 16.8°C and July high: 31.7°C and low: 21.3° C.)(Figs.2,3). Growth of *L. arvalis* occurred on agar medium amended with 1000 mg /ml CGA 173506. Similarly, growth of *T.harzianum* occurred on a medium amended with 1000 mg /ml of iprodione. These selections were stable through several transfers to media amended at the same fungicide concentrations and were used in the integrated control studies.Dusting of *Rhizoctonia*-inoculum preparation onto plants increased disease and reduced root weight (P = 0.05) compared to either the control or the plug inoculation technique (Table 2). There were no differences between the protective or therapeutic application of fungicides (Table 2). *T. harzianum* added as a soil amendment increased root length (4.6 cm)compared to the control (3.3 cm) (P=0.05). Integration of chemical and biological controls to decrease incidence of Rhizoctonia preemergence damping-off has been documented (17), however questionable mathematics and graphic presentation detracts from the research. Fortunately, the conclusion that integration of chemical and biological controls provides opportunities for enhancement and greater efficiencies in suppressing damping-off induced by *R. solani than either technique used alone* was valid. Other unique attempts at integration of biological agents have involved combinations with cultural techniques (tillage.) Success of integrating biological agents with cultural techniques to control diseases of snapbean caused by *Pythium* spp. and *R. solani* varied and depended on formulation and method of application of the biological agent. Preparations of biological control agents added in-

furrow or to seed were ineffective in reducing disease when used individually or in combination with cultural or chemical methods (15). In other experimentation (16), greater reduction of *Rhizoctonia* fruit rot was obtained by applying *T. harzianum* (WT-6) in conjunction with plowing than when either technique was used individually. We have shown that biological control agents selected for fungicide resistance can be integrated with those fungicides to enhance disease suppression and root growth. Prior research has shown that the fungicides benomyl, iprodione and mancozeb were effective in controlling web blight in greenhouse testing on large plants (13).

Web blight on Rosemary was first reported in 1992 (13) and was attributed to *R. solani* AG-1. The blight occurred on landscape plantings of the cultivar Prostratus, killing up to 80% of the branches. Our isolate OK-367, probably originated on field-grown plants used for propagation and is most severe on cuttings. Mist propagation of rosemary in the greenhouse provides ideal conditions for disease development and spread: warm temperatures and high humidities.

The difference in the success of integration with fungicides between *L. arvalis* and *T. harzianum* may be related to the higher concentration of fungicide applied in conjunction with *T. harzianum* compared to that used with *L. arvalis* and perhaps to the difference in the mode of action and efficacy between the two *L. arvalis* produces laetisaric acid (1) which is inhibitory to *R. solani* and *Pythium* spp. Our isolate of *T. harzianum* is a mycoparasite of *R. solani* (11) and is not known to produce fungal inhibiting

compounds.

Greater root weight of rosemary in *Trichoderma*-amended medium was similar to growth enhancement of broccoli seedling by both biocontrol fungi in previous experiments (5).

Amendment of potting mix with either biological control significantly reduced the incidence of *Rhizoctonia* blight compared to the control treatment (Figs.1,3) which indicates that control of the soil phase of the blight by the biological treatments can suppress total disease development during propagation of rosemary.

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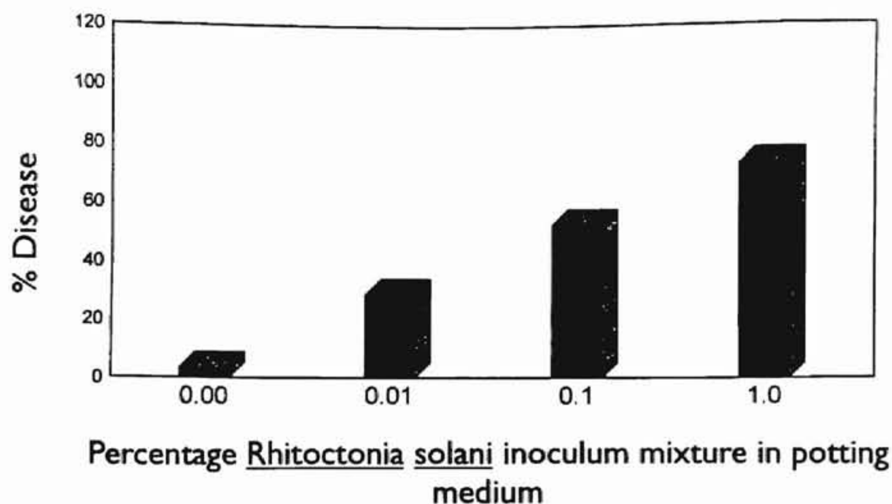

Fig. 1. Relationship between disease incidence of rosemary cuttings and the percentage of *Rhizoctonia solani* inoculum added (wt/wt) to the potting soil during May 1992 (4-week incubation).

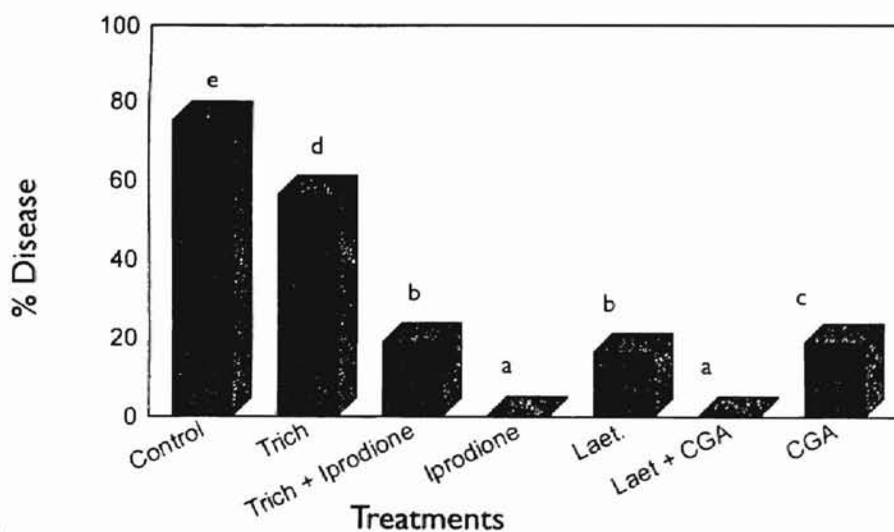

Fig. 2. Comparison of the effectiveness of chemical and biological controls, used alone or in combination, for Rhizoctonia blight of rosemary cuttings, May 1992. The density of *Rhizoctonia solani* inoculum mixture added to the potting soil was 0.01%. Biological control agents *Trichoderma harzianum* (Trich), 5.0 g of fermentation product/kg of potting soil, and *Laetisaria arvalis* (Laet), 5.0 g of dried sclerotia/kg, was added to potting soil before planting; and fungicides iprodione, 1.2 ml/473 ml of water, and CGA 173506, 0.035 g/473 ml of water, were applied to cuttings until runoff. Columns with different letters are significantly different ($P = 0.05$).

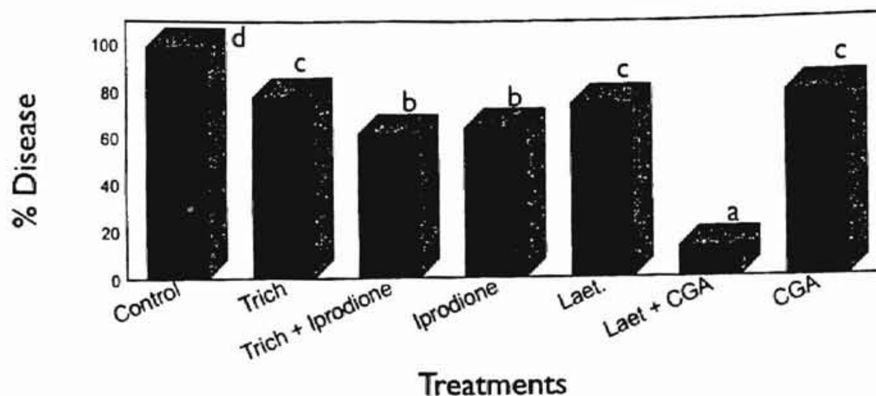

Fig. 3. Comparison of the effectiveness of chemical and biological controls, used alone or in combination, for Rhizoctonia blight of rosemary cuttings, July 1992. The density of *Rhizoctonia solani* inoculum mixture added to the potting soil was 0.01%. Biological control agents *Trichoderma harzianum* (Trich), 5.0 g fermentation product/kg of potting soil and *Laetisaria arvalis* (Laet), 5.0 g of dried sclerotia/kg was added to potting soil before planting; and fungicides iprodione, 1.2 ml/473 ml of water, and CGA 173506, 0.035 g/473 ml of water, were applied to cuttings until runoff. Columns with different letters are significantly different ($P = 0.05$).

Table 1. Comparison of techniques for inoculation with *Rhizoctonia solani*, with data pooled from two tests for all treatments for numbers of dead rosemary cuttings and root weight

Technique[v]	Dead plants[w] (no.)	Root fresh weight[x] (g)
Dusting, protectant[y]	4.92 a	0.59 a
Dusting, therapeutic[z]	4.88 a	0.60 a
Plug, protectant	1.75 b	1.17 b
Plug, therapeutic	1.25 b	1.26 ab
Control	0.33 c	1.60 c

[v] Dusting involved misting of cuttings with water and dusting 0.1 g of 0.1% *R. solani* mix onto the six cuttings in the six-pack. For the plug inoculation, a 1.0-cm disk was removed from an actively growing culture of *R. solani* on potato dextrose agar and placed on the surface of one of the end cells of the six-pack.

[w] Numbers are means of six plants with eight replications. Control received no inoculum; disease incidence is naturally occurring plant-to-plant spread. Means with different letters are significantly different ($P = 0.05$), Student-Newman-Keuls test.

[x] Weights are new secondary roots clipped from the main root and are means from six plants with eight replications.

[y] Fungicide sprays applied before application of *R. solani* inoculum.

[z] Fungicides applied after application of inoculum.

CHAPTER III

SAGE (*SALVIA OFFICINALIS* L.) PRODUCTION PRACTICES: HARVEST TIMING AND NITROGEN FERTILIZATION

Abstract

The objectives of this study were to determine the nitrogen fertilizer rate to optimize sage (*Salvia officinalis* L.) yield and to determine when to conduct the third and final harvest date in the fall for optimum yields and to insure winter survival. The first two mid-season harvest dates were identical for all plots. The first harvest was in May and the second harvest was in August. The four final late summer or fall harvest dates were 20 September, 11 October, 1 November, and 22 November. Timing of the initial harvest, the interval between successive harvests and the date of the last fall harvest are three very important factors to crop profitability and to the health of a perennial planting. Results indicate 20 September and 11 October should not be harvest periods for sage and that 1 or 22 November were the best periods of the year for a third and final harvest on sage. Nitrogen fertilization rates had little effect on yields or winter survival.

Introduction

Sage is a hardy perennial subshrub with flowers in whorls of four to eight at the axils. Flower colors can be pink, purple, white or blue and various intensities of each color. Leaves are opposite and up to two inches long. Leaf color is grayish green Plant height is 30 to 75 cm. Sage is hardy into the warmer parts of Canada. It is native to the northern Mediterranean coastal region. Soil should be slightly alkaline and sage will survive in low rainfall areas once the plant is established (Bailey and Bailey, 1976; Kowalchik and Hylton, 1987).

Sage is gaining in importance in its use for extraction of antioxidants. In a study of 32 spices done by Chipault *et al.* (1952) sage was reported to have antioxidants which were effective for use in protecting foods against oxidative rancidity. Chang *et al.* (1977) reported a patented process for extracting antioxidants from sage and rosemary. Presently, synthetic antioxidants are prominently used; however, the food industry is searching for natural souces of food preservatives due to worldwide interest. Some reports indicate low antioxidant activity in *Salvia officinalis* (Kim *et al.*, 1994). However, Djarmati *et al.* (1991) found that further purification of the alcoholic extracts from sage using supercritical CO extraction improved the antioxidant properties. The antioxidant activity was reported as being much higher than butylated hydroxytoluene (BHT) which is a common synthetic antioxidant. BHT is a white crystalline solid that is effective in animal fats but not as effective in vegetable oils (Dorko, 1994).

Nitrogen nutrition is an important factor affecting vegetative plant growth. However, there is little published information on nitrogen rates required for herbs (Ruminska, 1978; Simons, 1987). The majority of the herb literature states that too much nitrogen fertilizer results in lush growth with inferior quality due to low oil content. Adler *et al.* (1989) showed that the essential oil content of sweet basil (*Ocimum basilicum*) was decreased by 28% with a daily dose of ammonium nitrate. However, the effect of nitrogen on antioxidant activity is not known.

Materials and Methods

Dalmation sage was used which is not a variety but a type; therefore the plants are not uniform. To provide a uniform plant for this study, the sage plants were produced by cuttings taken from a superior line selected from a field of sage and rooted in a mist bench in the greenhouse. Field plant selections were based on upright plant type to facilitate machine harvest and vigorous plant growth. We also based the selection on an antioxidant rating received from a company which analyzed samples for antioxidant activity. The actual measurements of activity were not disclosed, only a rating system.

The rooted cuttings were transplanted into test rows in the field on May 14, 1990. The field experiments were conducted at the Vegetable Research Station, Bixby, Oklahoma, on a Severn fine sandy loam [coarse-silty, mixed (calcareous), thermic Typic Udifluvents].

Plots were 7.8 meters long with three rows one meter apart and the middle row was used for data collection. There were 26 plants per plot. The plots received 60 kg ha preplant nitrogen fertilizer. Plots were transplanted 14 May 1990. All plots were sidedressed with urea on June 1, 1990 at 20 $kg \cdot ha^{-1}$ nitrogen. Three experimental nitrogen treatments were 60, 120 and 180 $kg \cdot ha^{-1}$ nitrogen sidedressed (low, medium, and high fertilizer regime, respectively). The low, medium and high fertilizer plots each received 60 $kg \cdot ha^{-1}$ in April of each summer beginning in 1991. The medium and high fertilizer plots received another 60 $kg \cdot ha^{-1}$ in June and the high fertilizer plots received a third sidedress of 60 $kg \cdot ha^{-1}$ in July, thus reaching a sidedress total of 60, 120 and 180 $kg \cdot ha^{-1}$ for the year.

Plots were harvested when the majority of the plants were about to be in full bloom which was recommended by Foster (1966) for optimal quality. The middle row in each plot was harvested with a flail vacuum mower at a height of 15 cm which was determined in an earlier study by Motes *et al.* (1991). The harvested material was dumped onto a tarp and weighed in the field. A wet sample was taken at that time, weighed, dried with forced air at 49 °C, and re-weighed to determine dry mass yields per plot. Yields per hectare were then calculated according to the plot yield on a dry mass basis. A small sample was also collected at this time, air dried, and shipped to the cooperating company for quality analysis.

The four final harvest dates were 20 September, 11 October, 1 November and 22 November each year of the study. Only the fall treatment

harvest was made in 1990, the year of transplanting. In years following, the plots were harvested in May at the point prior to full bloom as stated above, again in August as was shown to be feasible (Motes and Bostian, 1990) plus the designated treatment harvest date, giving a total of three harvests per year. The interaction for sage yield between the nitrogen rate and the date of last fall harvest was also determined.

The statistical design was a randomized complete block with three replications and data were analyzed accordingly.

Results and Discussion

In 1991, yields were all high because it was the first year of regular harvest. However, on November 5, 1991, there was a hard, killing freeze (-8 °C) with no previous frosts to promote cold acclimation. The perennial vegetation all over Oklahoma was devastated. The plots with 20 September as the last harvest date lost 61% of the sage plants and the 11 October harvest date plots lost 8%. None were lost in the 1 November and 22 November harvest date plots. This indicates that harvest on 20 September disrupted plant processes which are necessary for winter survival. Note that the 1 November and 22 November harvest date plots' yields were unaffected in 1992 (Figure 1). However, the 20 September and 11 October harvest date plots' yields recovered somewhat in 1993 and were only slightly lower than both November last fall havest dates.

In alfalfa (*Medicago sativa*), a cut near the time of a killing fall frost will

increase the danger of winter injury and death of the plant. It was shown that alfalfa in Alabama needed six to eight weeks recovery time after harvest and before a killing frost. There are varying reports of the total nonstructural carbohydrate levels during different fall harvest dates and number of harvests. In alfalfa, frequent harvests do not allow the accumulation of enough total nonstructural carbohydrates between harvests which leads to stand decline (Ogg, 1988). Sage showed evidence of the same pattern of survival in reference to the date of the last fall harvest of the season. Some alfalfa harvest regimes recommend no later than mid-September or after a freeze. Foster (1966) suggests that the last cut on sage be in September to encouage overwintering and Kowalchik and Hylton (1987) state that sage cut after frost may cause winter kill.

Total fall harvest yields were significantly affected following the 1991 freeze. Figure 1 illustrates the significant loss in yield in the 1992 harvest season due to heavy plant losses in the plots of 20 September and 11 October fall harvest treatment which suffered 61% and 8% plant loss, respectively. Yields did not recover much in 1993 as there was an insignificant yield increase in the 20 September plots and a slight decrease in the 11 October plots indicating further plant loss in subsequent years following a freeze. However, the 1 November and 22 November plots were not directly affected. The drop in yields may be due to natural plant loss rather than freeze damage of 1991. Another year or two would need to be

evaluated in order to determine a field re-establishment time frame, if the yields continue to decrease each year.

Fall harvest date showed no significant difference in 1991 (Figure 2). There were significant differences in the 1 November and 22 November plots in 1992 and 1993 as shown in Figures 3 and 4. Yields from treatment harvests in 1992 and 1993 are significantly lower because of slower growth during the heat of the summer between August and September harvests.

By October, the plants were beginning to grow more vigorously. These data show that the most vigorous growth of the sage occurred between late September and early November. Embong *et al.* (1977) stated that sage grows best between the temperatures of 3 °C and 29 °C with the optimum temperature being 13 °C. This would support the data shown in Figures 3 and 4 with the optimum temperatures occurring in late September and into November. The 22 November harvest date yields were not significantly greater than the 1 November harvest date yields due to dormancy and winter dieback in 1992 as shown in Figure 4. According to Crockett and Tanner (1977), harvest should not be in early fall for Zones 6 and northward. Since Oklahoma is in Zone 7, there would be a small buffer. These data show that late fall is the time that plants have already gone into the semi-dormant stage of an evergreen, and harvesting at this stage depletes the plants' reserves. These data also indicate that there is very little growth after November because of the semi-dormant stage.

Yields in the 1 November and 22 November plots differed little from

year to year compared to the 20 September and 11 October plots (Figure 1). Combined yields for each year are shown in Figures 5, 6, and 7. Actual yields are listed in Table 1.

Nitrogen levels averaged over all harvest treatment dates showed no significant differences between years, nitrogen rates, or percent dieback after the 1991 freeze (Figure 8).

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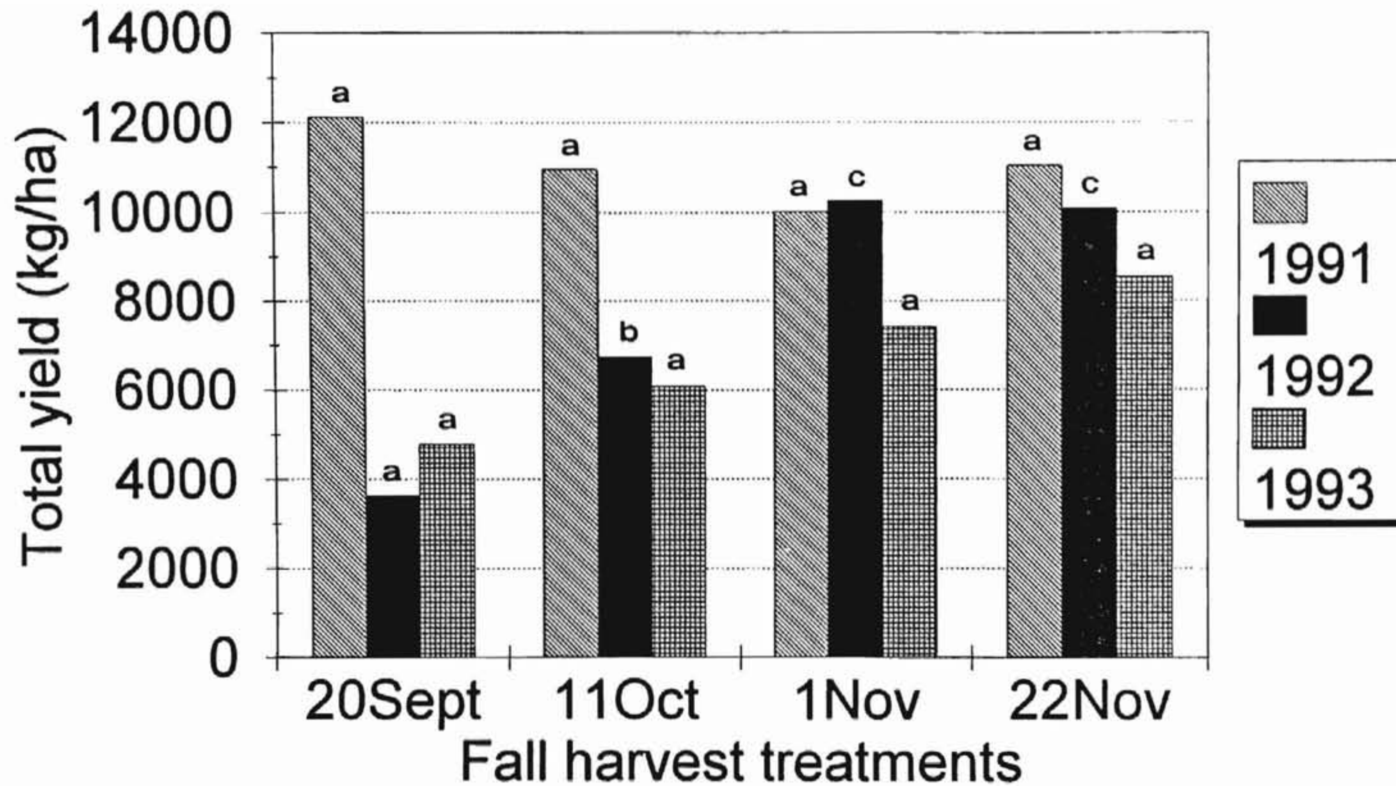

Figure 1. Combined yield (kg/ha) for each year of study by fall harvest treatments. Mean separation within years between fall harvest treatment by Duncan's new multiple range test, $P \leqslant 0.05$.

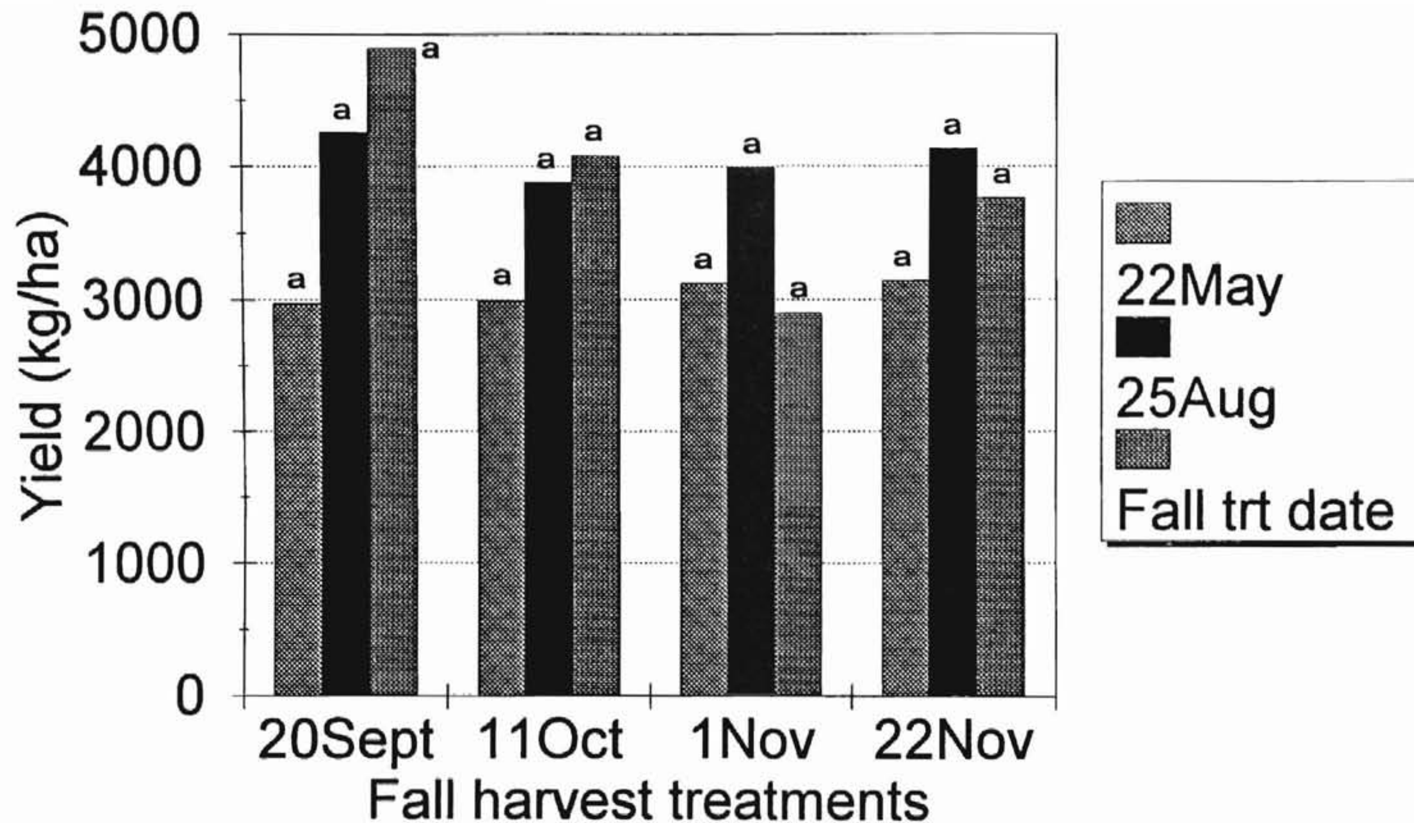

Figure 2. Yield (kg/ha) by individual harvest for each fall harvest treatments, 1991. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leq 0.05$.

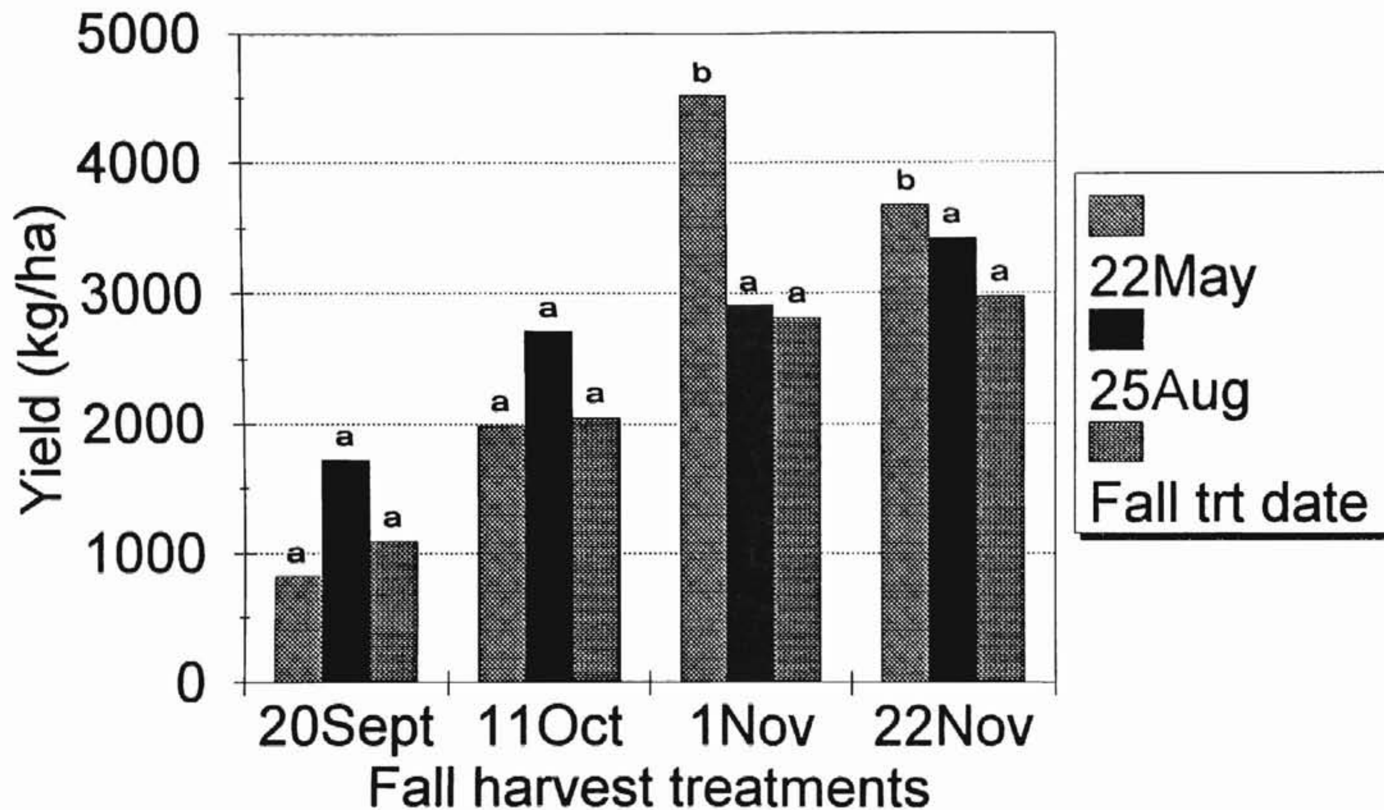

Figure 3. Yield (kg/ha) by individual harvest for each fall harvest treatment, 1992. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leqslant 0.05$.

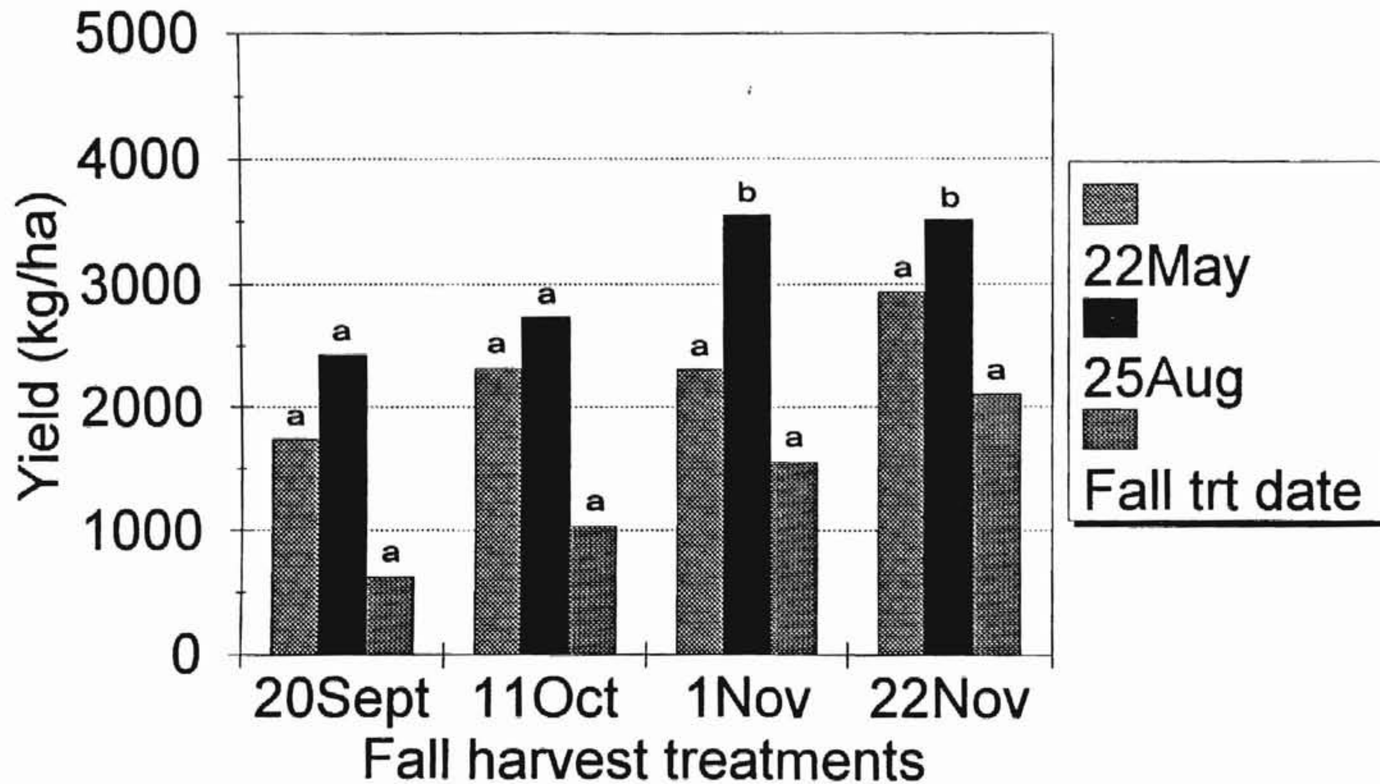

Figure 4. Yield (kg/ha) by individual harvest for each fall harvest treatment, 1993. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leqslant 0.05$.

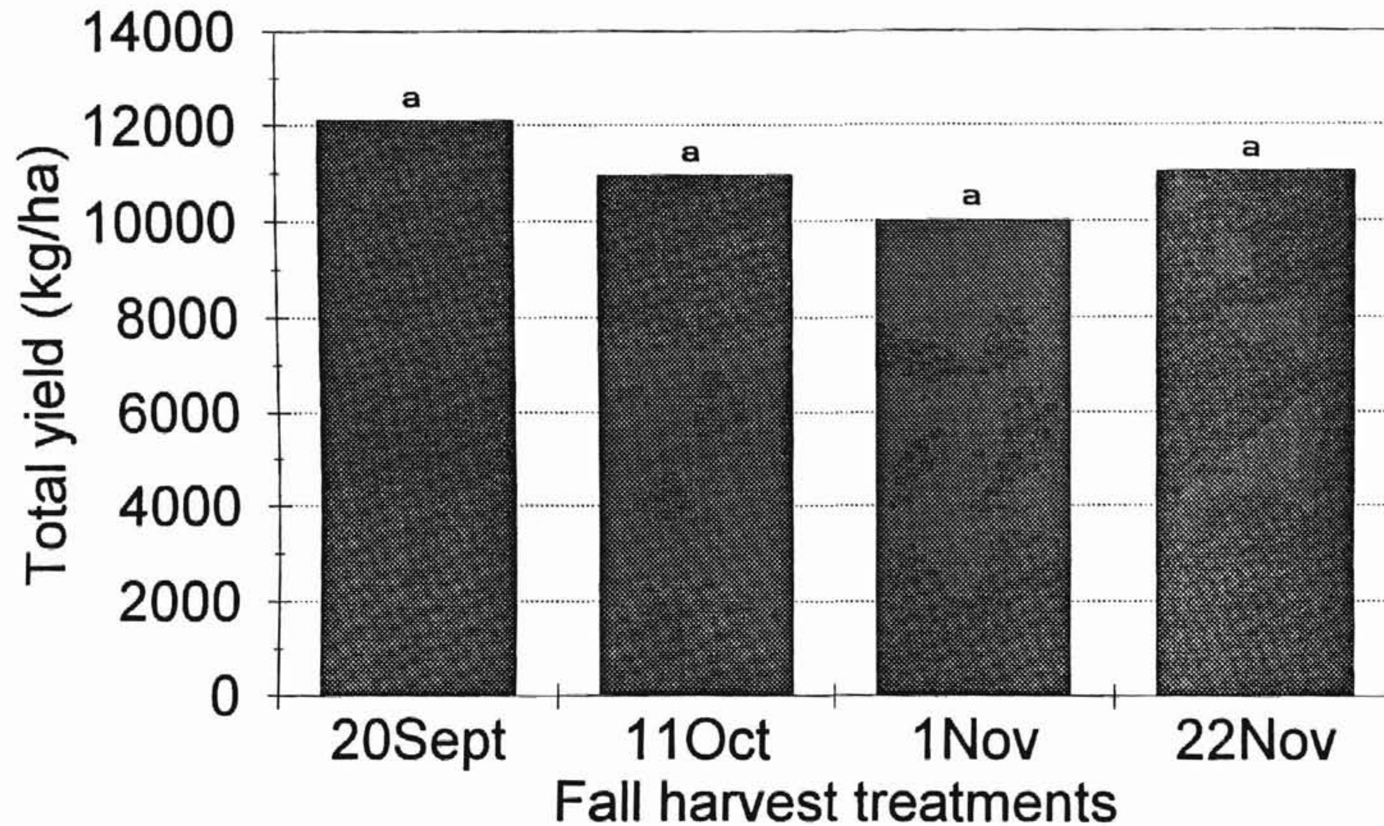

Figure 5. Combined yield (kg/ha) over all three harvests of the season, 1991. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leqslant 0.05$.

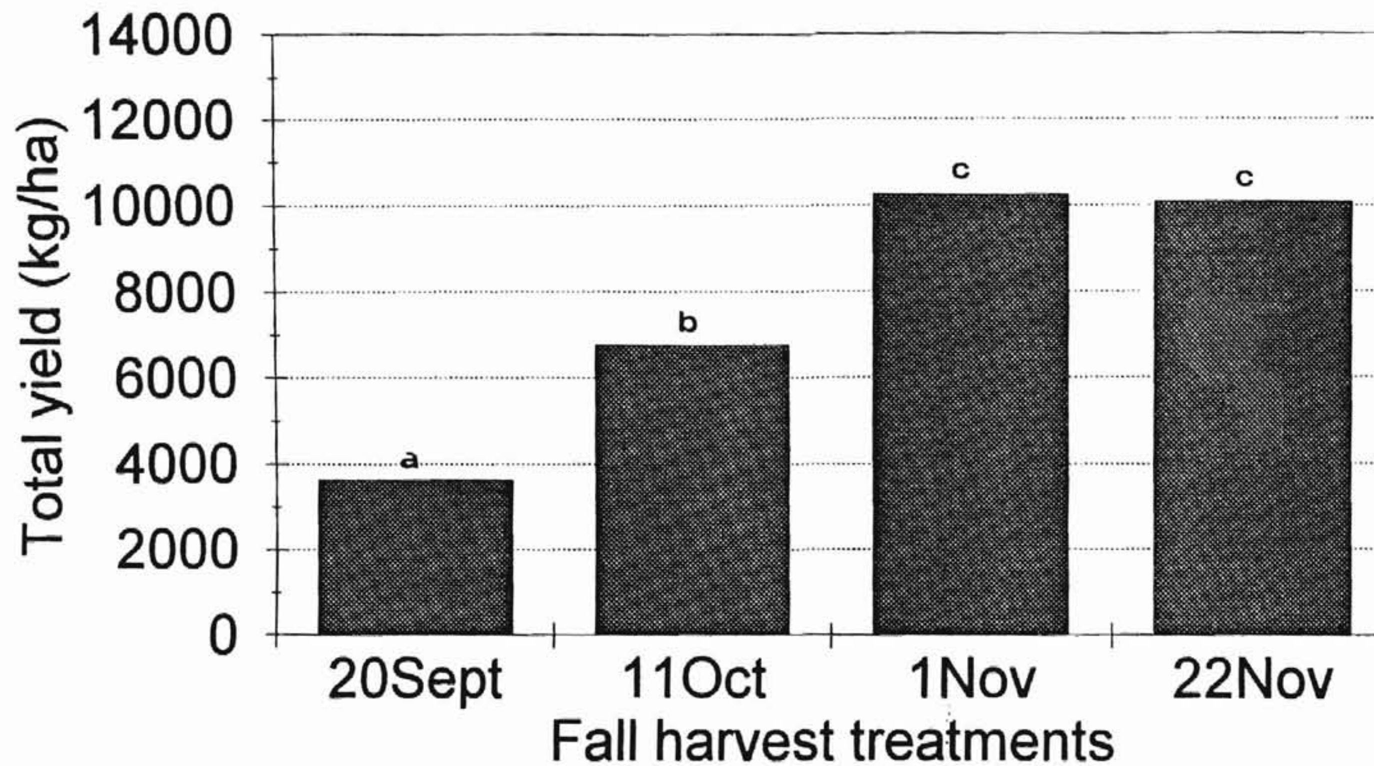

Figure 6. Combined yield (kg/ha) over all three harvests of the season, 1992. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leqslant 0.05$.

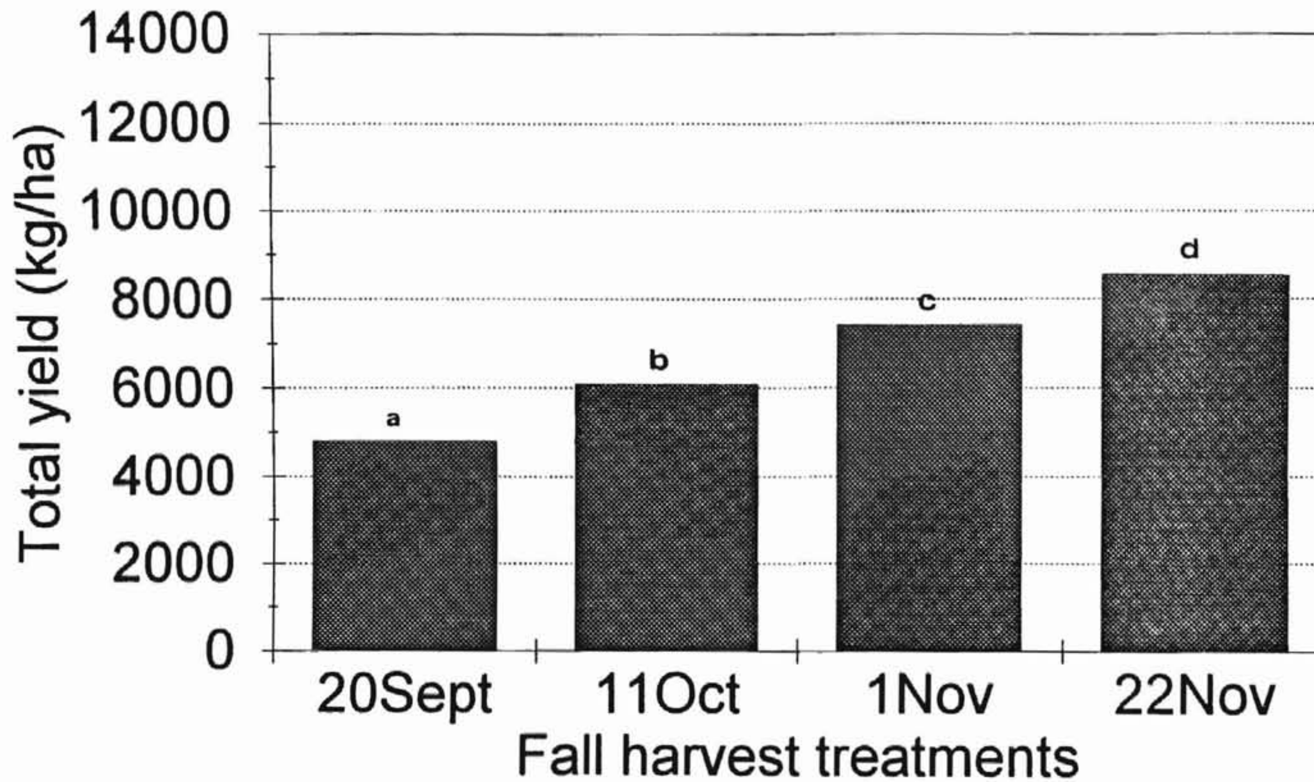

Figure 7. Combined yield (kg/ha) over all three harvests of the season, 1993. Mean separation between fall harvest treatments by Duncan's new multiple range test, $P \leq 0.05$.

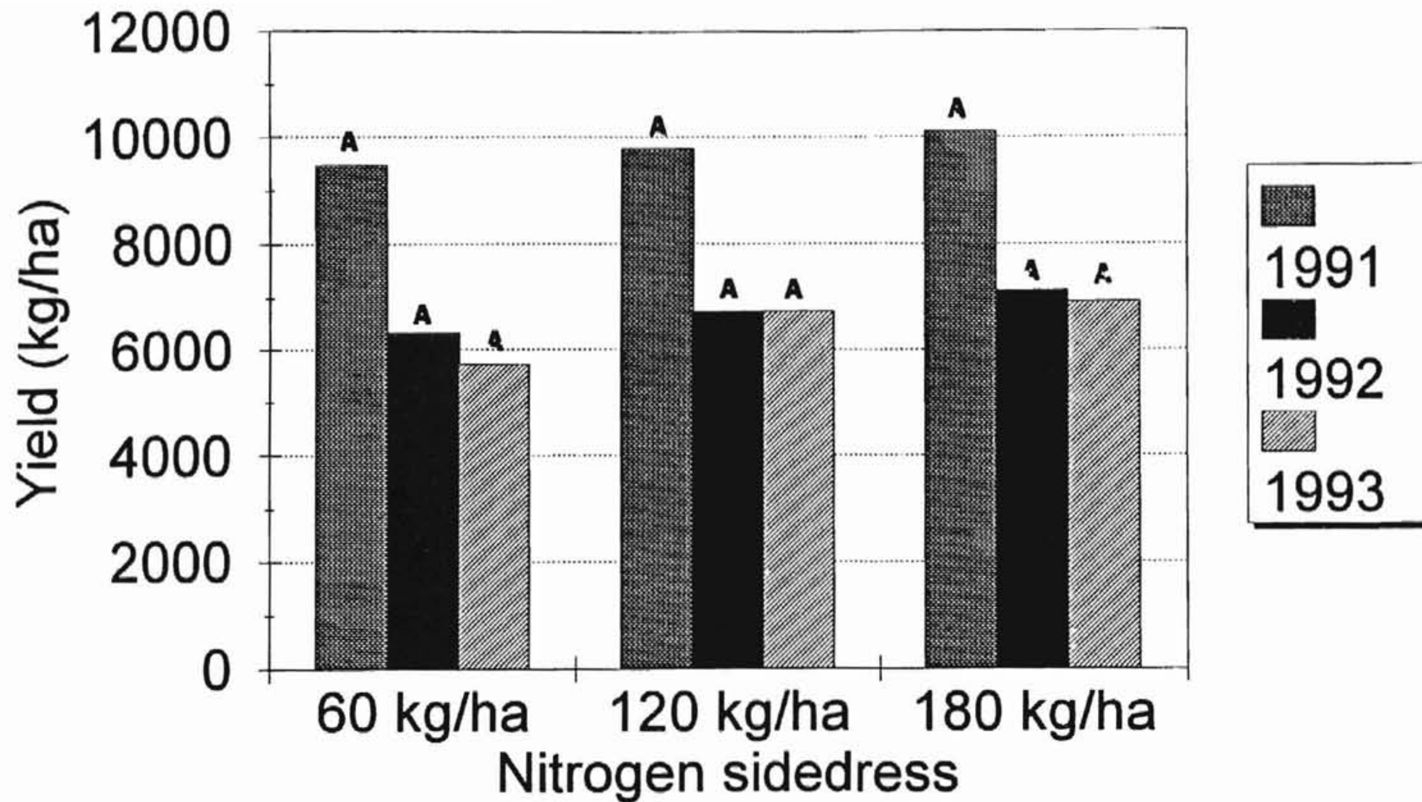

Figure 8. Total yield (kg/ha) averaged over all four fall harvest treatments. Mean separation within years between nitrogen sidedress treatments by Duncan's new multiple range test, $P \leqslant 0.05$.

Table 1. Sage yields (kg/ha) from each harvest by year and fall harvest treatment.

Year	22May	25Aug	20Sept^{z}	11Oct	1Nov	22Nov	Total
	Approximate harvest dates						
1991	2974	4261	4887				12122
	2994	3882		4085			10961
	3126	3991			2900		10017
	3145	4136				3760	11041
1992	821	1714	1090				3625
	1980	2714		2055			6749
	4517	2917			2812		10246
	3673	3421				2980	10074
1993	1737	2427	628				4794
	2307	2735		1039			6081
	2306	3555			1561		7442
	2939	3513				2102	8554

^{z}Treatment harvest dates are approximate. Each plot was harvested three times during the season, 2 regular harvest plus one treatment harvest.

VITAE

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