VIRGINIA COMMONWEALTH UNIVERSITY
Department of Biology

Richmond, Virginia 23284-2012 (804) 367-1562

Plant Sciences

Mr. Halvard Alexander
J & J Agri-Products & Services, Inc.
220 South Second Street
Dillsburg, PA 17019

Dear Mr. Alexander:

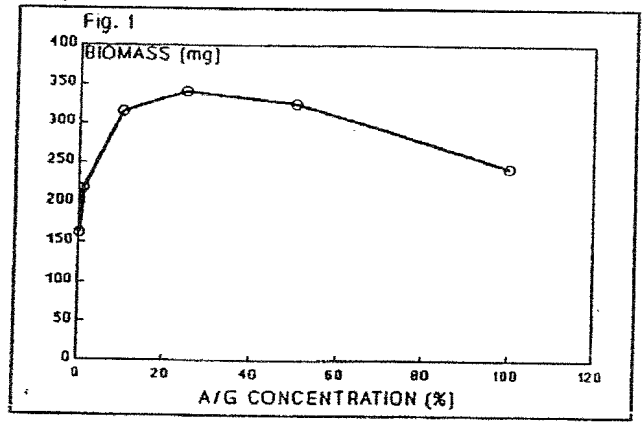
This letter summarizes the progress we have made on determining the best dose of your product A/GJA-F90 (A/G) for use in a bioassay kit. We have in the process discovered a very interesting property of your compound; it can influence the shoot to root partitioning. We found that as you increase the concentration of your compound, the total biomass increases. However, at the same time, the root biomass (as measured via length) decreases. Together this would indicate that your product can alter the shoot/root ratio in favor of shoot. It should be noted, however, that the shorter roots in A/G treated plants had an increased amount of lateral root differentiation. This would indicate that treatment of lettuce with your compound should lead to more vigorous seedling development. I think that this is a very important discovery.

Procedures:

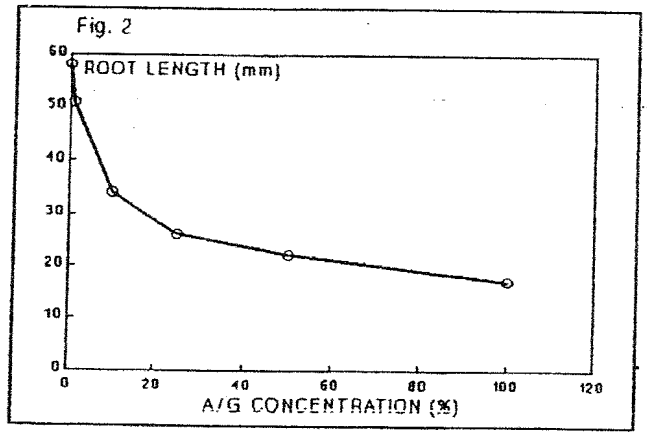
The same lettuce seed cultivar was used in this study as in previous studies. The A/G compound was used at 100, 50, 25, 10, 1, and 0 %. A/G was diluted using mixed bed deionized water and each dilution was adjusted to a pH of 6.0. Ten lettuce seeds were placed per 60x15 mm petri dish on Whatman filter paper disks saturated with the appropriate dilution. The dishes were then incubated at 25C for 7 days under cool white fluorescent lights (6000 lux). At the end of the incubation time the total biomass was determined and the root length on ten representative plants was measured for each dilution level. All treatments were run in triplicate using ten seeds per treatment (total N per dilution therefore was 30 plants; for a total of 180 plants).

Results:

Treatment with A/G caused a significant increase in total biomass (Fig. 1). The optimum concentration was 25% with the 50 and 100% dilutions still higher than the control.



Treatment with A/G caused a significant reduction in primary root length (Fig. 2) although their was a increase in lateral root differentiation.



Conclusions:

It is clear from this study that your compound can stimulate growth and biomass increase in lettuce. Under our experimental conditions, the stimulatory activity could be seen at a concentration as low as 1%. The optimum concentration, at least in this study, was 25%. Even at 100%, activity was greater than the control; no toxic response was detected. We will repeat this study several times and will try to better pin point the optimum concentration near the 25% level. It was also clear from this study that your compound can alter root development. Treated plants had shorter roots, even at 1%, but had more lateral root

development. Your compound , at least with lettuce, can apparently alter shoot/root developmental patterns and in this case result in more vigorous seedlings. Again there was no toxic effect on the root. In future studies we will continue to monitor these root responses. The possibility that your compound can alter tissue development patterns and partitioning is really interesting.

Sincerely,

R W Fisher

Robert W. Fisher, Ph.D.
7-3-91

ps:

1. We will start pH experiments next

BIOASSAY FOR GROWTH PROMOTING ACTIVITY IN J&J PRODUCTS R/ONM-J91 AND A/GJA-F90

ROBERT W. FISHER, Ph.D.
Department of Biology
Virginia Commonwealth University
Richmond, Virginia 23284-2012

ABSTRACT

The growth promoting activities of J&J (Dillsburg, PA, USA) products R/ONM-91 and A/GJA-F90 were tested using lettuce (Lactuca sativa) seedlings. Seed germination was allowed to take place in 10% dilutions of each product adjusted to pH 6.0. Seedlings were allowed to grow in these solutions for 5 days. At the end of 5 days, the seedlings were weighed and their biomass increases compare to each other and to the water solvent control. Both products were found to be significantly more active than the water control.

INTRODUCTION

These studies were undertaken to test for phytoactive growth promoting activities in two J&J product formulae (R/ONM-91 and A/GJA-F90). This was done as a continuing effort to monitor growth promoting activity in J&J products and to establish a data base foundation for future studies on these compounds.

MATERIALS AND METHODS

Plant material: Lettuce (Lactuca sativa) seeds (fruits) were obtained from the USDA Plant Growth Laboratory in Beltsville, Maryland. They were stored at -20C until needed.

J&J Product: Product was hand delivered by Halvard Alexander of J&J Agri-Products on 2-9-91. R/ONM-J91 was orange in color, had a significant sediment and a pH of 4.3. A/GJA-F90 was yellow , had less sediment and a pH of 3.7. Each product was shaken well and diluted to the 10% concentration level using VCU mixed bed deionized water and adjusted to pH 6.0. The VCU water was also used as the control (blank).

TREATMENT PROTOCOL: Whatman #3 filter paper disks (9.0 cm) were placed into Falcon Petri dishes (100 x 15 mm). The filters were totally saturated with the test solutions. All treatments were run in triplicate. Ten lettuce seeds were added to each treatment dish and after sealing with Parafilm, the dishes were placed under cool white fluorescent light (35 umol/m^2/s). After 5 days of culture, the seedlings were removed and weighed, collectively per treatment dish, to the nearest milligram.

LETTUCE SEED BIOASSAY PROTOCOL

PLANT: Lactuca sativa var. Grand Rapids.
USDA Light and Plant Growth Laboratory
Beltsville, MD

CONDITIONS: Temperature 22-24 C
Continuous light (24:0 photoperiod)
3,000 Lux
Cool white fluorescent

PROTOCOL:

1. Prepare all samples (Nitromax etc.). Dilute to the appropriate concentrations (i.e Nitromax at 10% v/v). Negative control will be the water source used for dilutions. Positive control, if used, could be 10 to the minus 6 molar cytokinin (Kinetin).

2. Adjust all sample solutions to pH 6.0.

3. Use sample solutions to moisten (to saturation) Whatman number three filter disks held in 60 mm Petri plates.

4. Set up three plates per treatment (thus in triplicate). Place 10 lettuce seeds on each plate (N = 30 per treatment).

5. Daily observations (every 24 hours) should be made for each plate to determine germination and seedling growth characteristics.

6. After 5-7 days of growth, harvest the whole seedlings and weigh their total biomass (to the nearest mg) per plate using an analytical balance.

7. Determine means for each treatment and compare these using either ANOVA or t-Tests.

RESULTS

Both R/ONM-J91 and A/GJA-F90 promoted growth in the lettuce seedling when compared to the VCU water control (Fig. 1). Each treatment dish containing J&J product showed a growth stimulation significantly greater than the water control (Table 1).

TABLE 1 J & J Product Response

	(mg Biomass Increase)				
SAMPLE	DISH 1	DISH 2	DISH 3	AVERAGE	sd
VCU water	217	180	187	194.7	19.7
R/ONM-J91	288	348	288	308.0	34.6
A/GJA-F90	335	333	263	310.3	41.0

DISCUSSION

This study has shown that products R/ONM-J91 and A/GJA-F90 both have phytoactive growth promoting activity. We will use the A/GJA-F90 product for future studies designed to define specific bioassay conditions for test kits to be supplied to interested clients.

Report Submitted: 5-30-91
RW Fisher
VCU Biology

Fig. 1

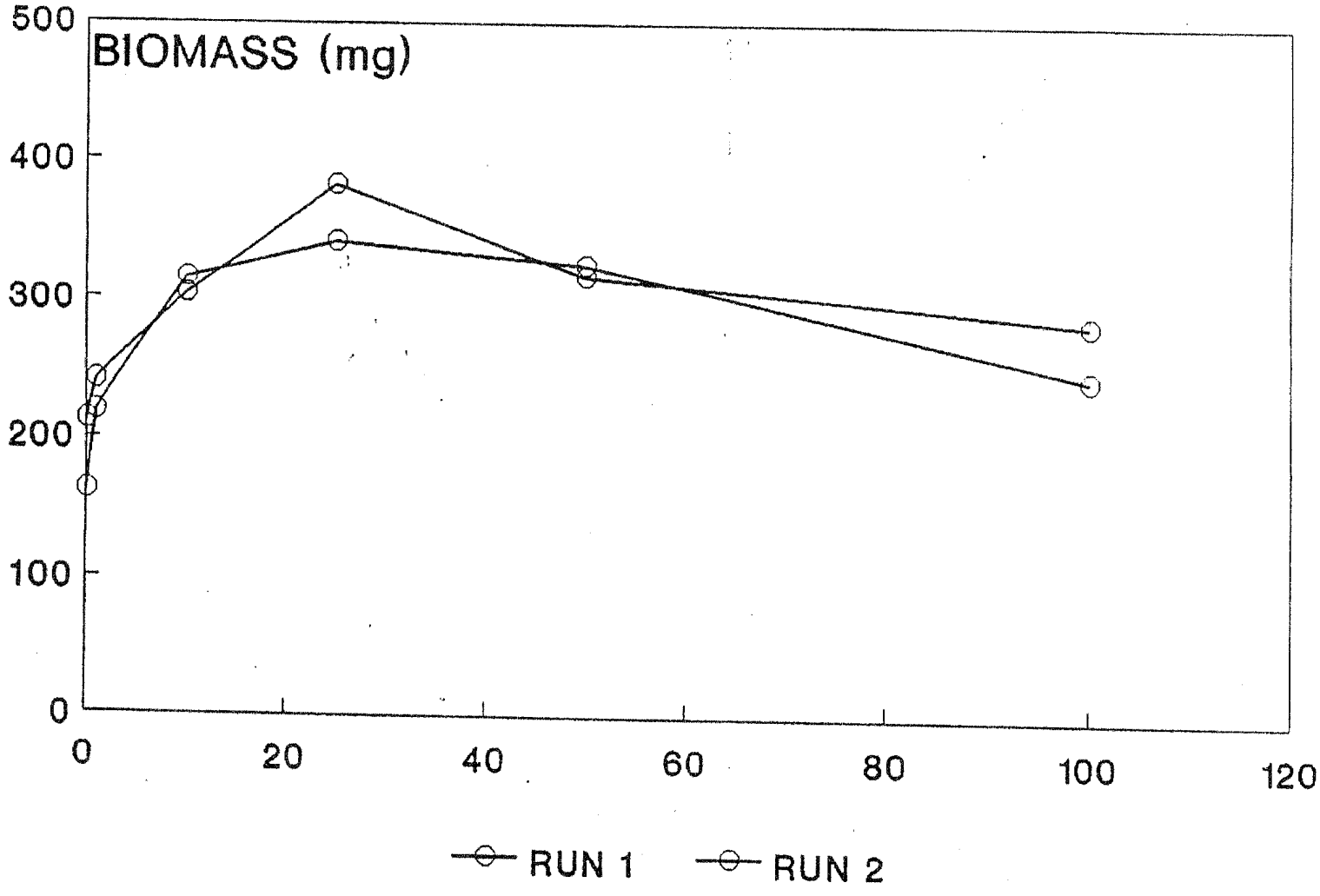

BIOMASS (mg)
500
400
300
200
100
0
0
20
40
60
80
100
120
RUN 1
RUN 2
A/G CONCENTRATION (%)

Fig. 2

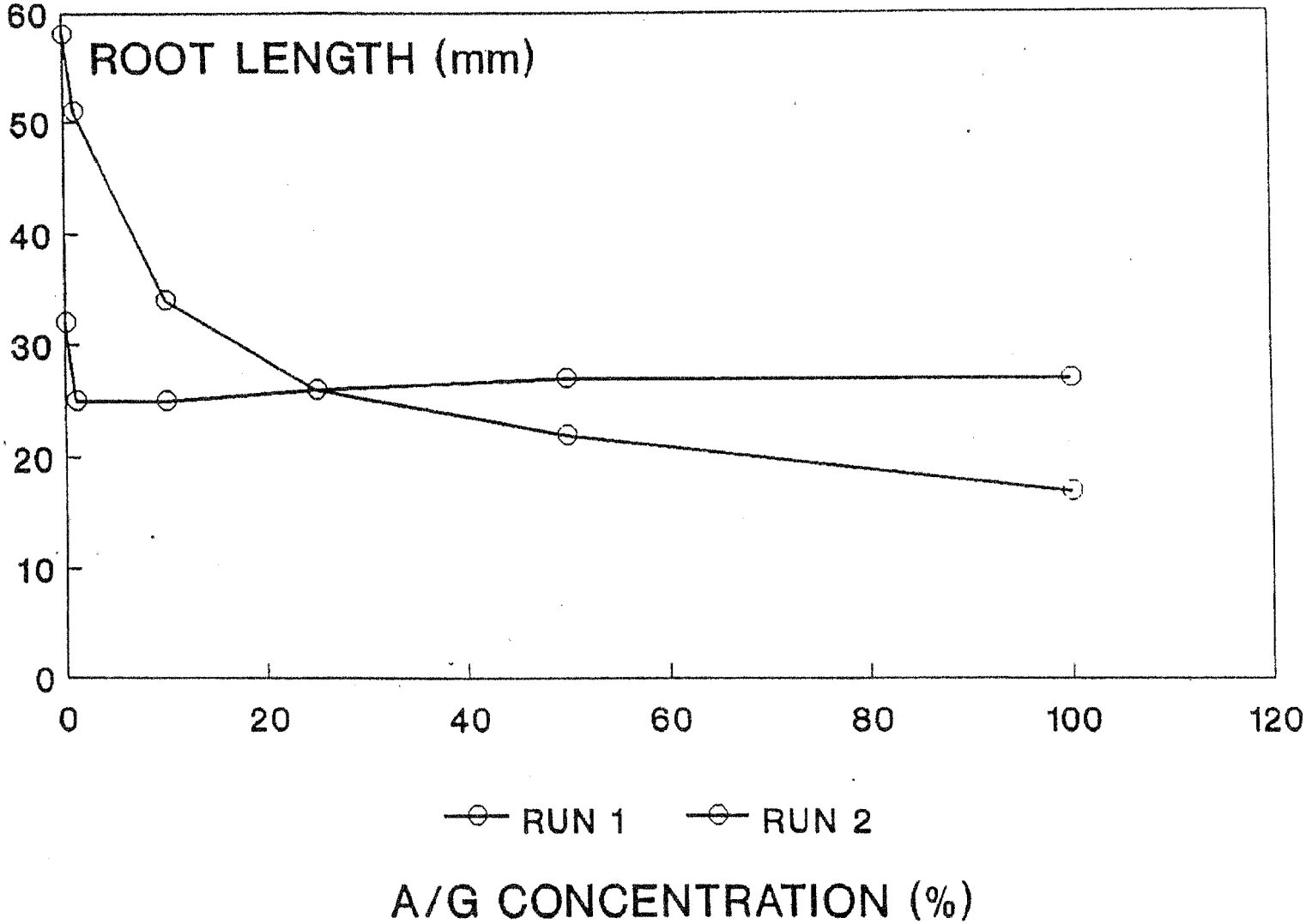
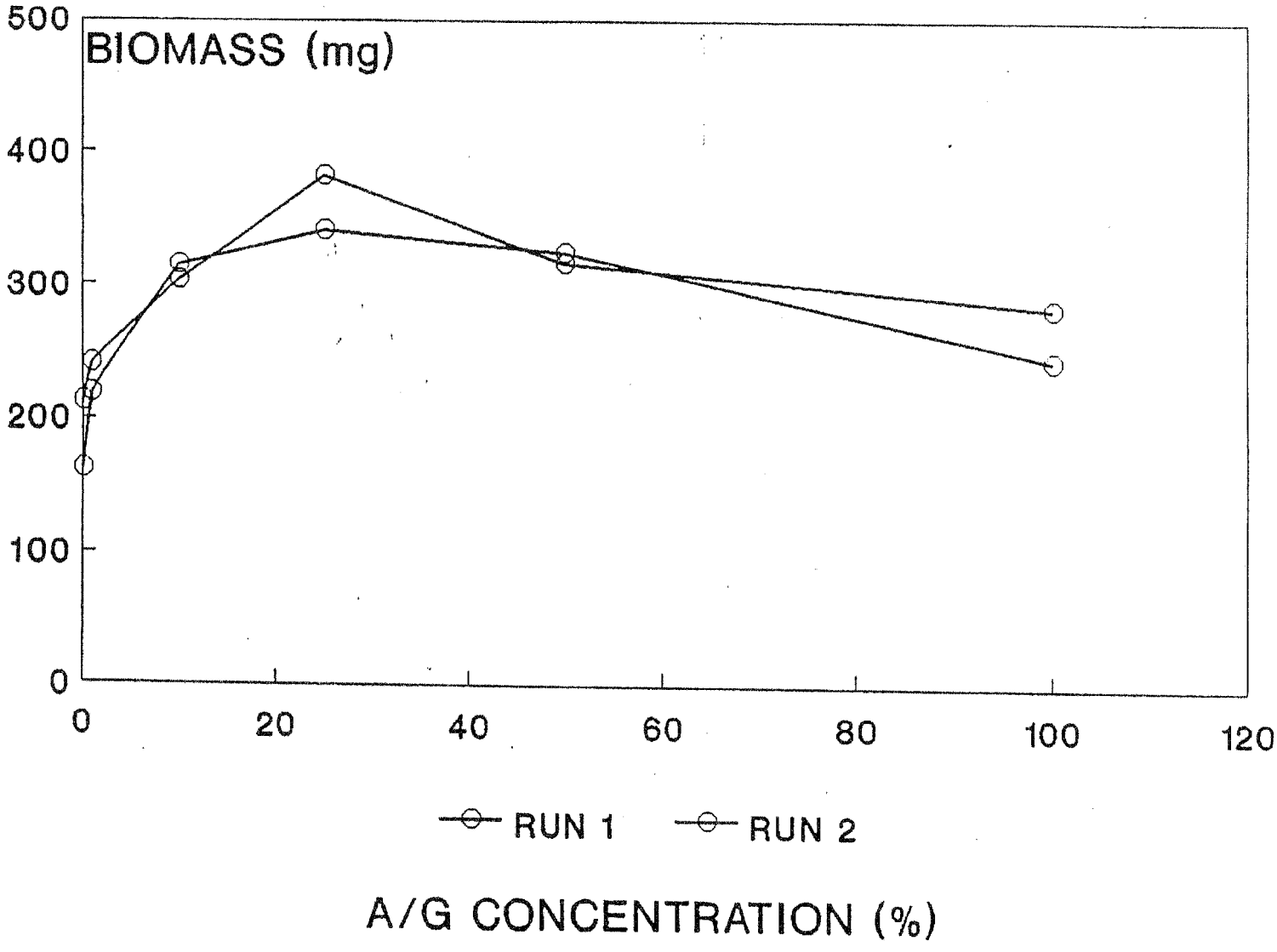

ROOT LENGTH (mm)
60
50
40
30
20
10
0
0
20
40
60
80
100
120
RUN 1
RUN 2
A/G CONCENTRATION (%)

Fig. 1


BIOMASS (mg)
500
400
300
200
100
0
0
20
40
60
80
100
120
RUN 1
RUN 2
A/G CONCENTRATION (%)

REPORT

BIOMONITORING OF NITROMAX ACTIVITY
A SIMPLE PROTOCOL

ROBERT W FISHER

DEPARTMENT OF BIOLOGY
VIRGINIA COMMONWEALTH UNIVERSITY

JULY 27, 1989

ABSTRACT

This report summarizes the work that has been completed during the past three years by Dr. Robert W. Fisher and his research group at Virginia Commonwealth University in Richmond, Virginia on the product Nitromax which is produced by J&J AGRI-PRODUCTS & SERVICES, INC. of Dillsburg, Pennsylvania. This report is for the sole use of J&J and any other use should have their prior approval. During the past three years, we have shown that Nitromax has cytokinin-like bioassay activity and have developed a simple but sensitive lettuce seedling bioassay to monitor bioactivity in Nitromax.

INTRODUCTION

Several years ago, J&J asked our group to study their product Nitromax to help determine the bases for its bioactivity. In doing these studies, we found that their formulation showed cytokinin-like activity as displayed in the radish cotyledon expansion bioassay. This assay is not very easy to run on a day to day basis and therefore, we developed a relatively simple bioassay using lettuce seedlings to monitor for similar activity. The purpose of this report is to explain the protocols involved in this new assay and to review some of the results that we have obtained using it with a number of Nitromax samples.

MATERIALS AND METHODS

Plant Material:

We used lettuce (Latuca sativa var. Grand Rapids) obtained from the USDA Light & Plant Growth Lab in Beltsville, Maryland. These seeds were stored at 8C in the refrigerator and brought to room temperature when needed for an experiment.

Growth Conditions:

Seeds were germinated and seedlings were grown in the light (3,000 lux cool white flourescent light) at 22-24C. These plants were monitored daily and additional treatment solution added as needed.

Treatment Protocol:

Following J&J's instructions, we ran all of our lettuce assays with the Nitromax concentrations at 10%. All experiments were run blind; that is, J&J sent samples in numbered containers (1,2,3...or A,B,C...) some of which may have been water blanks. Therefore, we did not know the nature of the formulations in each sample. This was done to make sure that our results were not biased and was done at our suggestion. After receiving the samples, 10% solutions were made by diluting the samples with an appropriate amount of distilled water (v/v).

Distilled water was run as a negative control and on some occasions, kinetin (10 to the -6 molar in water) was included as a positive control.

Experimental test runs were set up using 60 mm glass Petri dishes and Whatman #1 absorbent filter paper disks. The pH of each 10% sample was adjusted to 6.0. Solutions were then transferred to the filter paper disks held in the glass Petri dishes. Enough sample volume was transferred to saturate the filter paper disks. Ten lettuce seeds were placed on top of the saturated filters and allowed to germinate and grow under the conditioned described above for 5-7 days. At the end of the growing period, the total seedling biomass was determined (to the nearest mg) using a Sartorius analytical balance. All treatments were run in triplicate and the means plotted against sample number.

RESULTS

The biomass data for six separate assay runs are shown in Fig. 1-6. In all cases, the water control (VCU) was less than the kinetin (KIN) positive control. Also in every case where a J&J sample was significantly below the other samples (Fig. 1, sample 2; Fig. 2, sample 3; Fig. 3, sample 4;& Fig. 4, sample 4) it was later identified by J&J as their water blank. In Fig. 5 none of the J&J samples were water and in Fig.6 we would hypothesize that sample C was their water control (this has not yet been verified).

verified by JMA 8/5/89

DISCUSSION

The bioactivity of active Nitromax samples from J&J can be easily determined using the lettuce seedling monitor protocol. In the radish cotyledon expansion assay, the cotyledon tissue expands in the presence of kinetin and active Nitromax (Fig. 7, RT; R= not treated with Nitromax). A similar response is seen with the lettuce seedlings (Fig. 7, LT; L= not treated with Nitromax). Also note that lettuce seedlings treated with Nitromax (10%) show a significant expansion of the cotyledon tissues in addition to a stimulated secondary leaf growth and differentiation (Fig. 7, LT and arrow).

We conclude that the lettuce assay system can be used to test for Nitromax bioactivity and that the Nitromax formulation has a significant positive effect on plant growth and development.

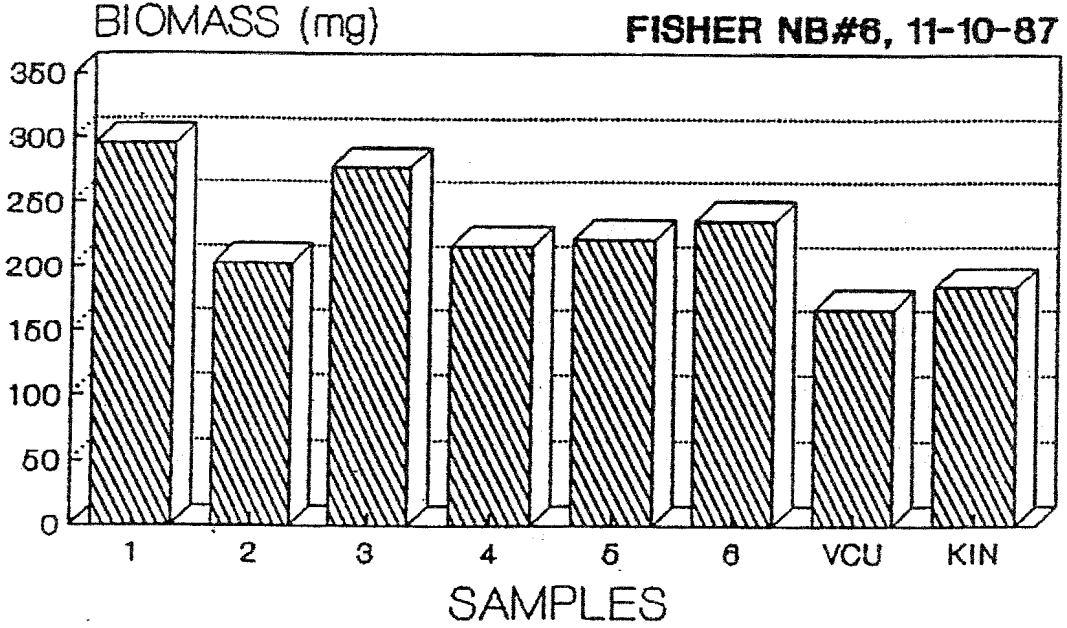

BIOMASS (mg)
FISHER NB#6, 11-10-87
350
300
250
200
150
100
50
0
1
2
3
4
5
6
VCU
KIN
SAMPLES

FIGURE 1