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Bacterial effects on the growth of Genovese Basil.
Final Report to S.A.R.E.

In the herb industry, the aesthetics of plants is very important. Plants need to be full and healthy. During winter months when the quality and quantity of sunlight diminishes, so too does the appearance of many greenhouse herb crops. Our goal was to find a viable method of increasing lateral branching in edible herb plants in order to replace the current labor intensive method of removing apical meristems. Cytokinins are a class of hormones which have proven effective at releasing lateral buds. Use of synthetic versions of this hormone has proven effective on ornamental crops but is not approved by the FDA for use on food crops. This led us to question whether or not it might be possible to use hormones produced by biological means (e.g. Soil Bacteria). We thus designed an experiment applying sprays of various bacteria, known to produce cytokinins, and their metabolites, to trials of Genovese Basil plants.

Since reception of the SARE grant my co-sponsor, Bioshelters, has erected a new acre large, state of the art, domed greenhouse, housing it's symbiotic herb and fish cultivation operations. This has increased Bioshelter's production capacity significantly as well as their need for a more effective method of maintaining desirable plant morphologies.

My main collaborators were Dr. Lyle Craker and Dr. William Torello of the Plant and Soil Science department at U. Mass Amherst. They were both instrumental in providing key advice and information concerning the experimental design, the various protocols, access and use to equipment and the general interpretation of the data.

Three species of soil bacteria known to produce cytokinins were grown and applied to isolated trials of twelve Genovese Basil plants each. They were compared against applications of control of water and three concentrations of a kinetin (synthetic cytokinin) solution,... 10mg/L 15mg/L and 20mg/L. The bacterial species used included Rhizobium leguminosarum, Cornybacterium fascians, Agrobacterium gluteans. Pseudomonas aeruginosa was discontinued due to it's potential as a lung infectant.

After attaining optimal population densities of each bacteria the whole broth was sprayed onto the corresponding hydroponically grown basil plants, three times a week every other week. Small quantities of

application of the bacteria and it's metabolites over the plant. This protocol was repeated several times with results being collected at the harvest of the plants in their sixth week of growth. Conclusions were based upon data consisting of plant height, lateral branch length, average distance between internodes and dry leaf mass.

The concentrated kinetin applications did not produce the intended lateral branching, this leads us to question whether the cytokinin route is still the way to go. This was an oversight on my part. It would have made more sense to test kinetin first to see if it produced the desired results. In fact, the ideal scenario would have had me testing all the pre-existing commercial products, for inducing lateral branching in flowers, on herb plants. Based on those results, I could have looked for possible biological means of mimicking the proven active ingredients

Unfortunately the results, even with the multiple replications, contained too much variability to be statistically significant. Within the various trials, differences in plant growth seemed to be due more to variables of pest damage, germination rate, and genetic variability between seeds than by the actual applications themselves. This was evident when compared to the control. Even the kinetin applications did not produce the lateral branching intented and actually seemed to stunt overall plant growth.

A couple of factors may be related to why the applications failed to significantly effect plant growth. One, there may not have been high enough concentrations of the cytokinins produced by the various bacteria to have an effect,... and two, the cytokinins, if sufficient, may not have been able to penetrate the plant's cuticle in order to effect bud growth. A follow up experiment might deal with increasing concentrations of the broths and using other forms of application media.

There is still room to continue with the possibility of effecting bud growth via bacterial metabolites Future experimental designs might incorporate clonal tissue culture in order to eliminate such variables as genetics, disease and germination rates. Although this approach would not accurately mimic field environments, it could be useful in ascertaining initial effectiveness. It also might prove useful, as mentioned earlier, to somehow concentrate the bacterial broths and or apply them using lipid based solvents.

This report has been given to the U.Mass agricultural extension office for future reference by others interested in the subject of microbiological plant regulation.

Bacteria Application Results
*See bottom of page for key

APPLICATION	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
AG FOLIAR 1	cm	cm	cm	grams
1	33	8	1.5	1.11
2	33	5.5	5.5	1.5
3	21	3.5	2.3	1
4	23	5	2.3	1.01
5	23	8	2.3	0.83
6	22	4.5	3.5	1.67
MEAN	25.83	5.75	2.90	1.19
STD. DEV.	5.60	1.86	1.43	0.33

AG FOLIAR 2	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	31.5	5	3.3	1.59
2	32	5.5	4.5	1.15
3	31	6	4	1.49
4	23	3	2.75	1.17
5	25	4	2.5	0.88
6	23	3.5	3	1
MEAN	27.58	4.50	3.34	1.21
STD. DEV.	4.36	1.18	0.77	0.28

RL FOLIAR 1	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	27	5.5	3	1.13
2	27	4	2	1.45
3	34	4	4	2.7
4	21	5	2	0.53
5	25	6	3.25	1.14
6	32	4	3.25	1.93
MEAN	27.67	4.75	2.92	1.48
STD. DEV.	4.72	0.88	0.79	0.75

RL FOLIAR 2	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	24.5	3.5	2	1.4
2	33	6	4	1.59
3	23	4	3	1.25
4	23.5	5.5	2	1.48
5	16.5	4	0.75	0.69
6	27	5	4.25	1.72
MEAN	24.58	4.67	2.67	1.36
STD. DEV	5.40	0.98	1.34	0.36

AG-Agrobacterium gluteans
RL-Rhizobium leguminosarum
CF-Cornybacterium fascians
PL.- Plant
I NODE- Internode
LAT. BRANCH- Lateral branching.
WGHT-Wieght
STD. DEV.- Standard Deviation

Bacteria Application Results
*See bottom of page for key

CF FOLIAR 1	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	24	6	3.25	0.93
2	28	5	2.5	1.28
3	26	6	1.5	0.93
4	31	5	3.5	1.11
5	16	2.5	1.25	0.54
6	19	4	1.25	0.76
MEAN	24.00	4.75	2.21	0.93
STD. DEV.	5.62	1.33	1.02	0.26

CF FOLIAR 2	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	22	3	1.5	1.23
2	28	5.5	2	
3	25	4	4	1.07
4	36	6	2.25	2.03
5	24	3	1	
6	30	3.5	2	1.23
MEAN	27.50	4.17	2.13	1.39
STD. DEV.	5.05	1.29	1.02	0.43

CONTROL	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	28	4	2.75	1.65
2	28	6	3.25	1.32
3	25	5	2.5	0.77
4	22	4	2	1.03
5	35	5	3.5	1.38
6	35	7	6.5	2.23
MEAN	28.83	5.17	3.42	1.40
STD. DEV.	5.27	1.17	1.60	0.51

Kinetin 10mg/L	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	22.5	3	4	0.71
2	15	1.5	5	0.39
3	34	6	10	0.75
4	26	3	5.75	1.53
5				
6	17	2	3.5	0.38
MEAN	22.90	3.10	5.65	0.75
STD. DEV.	7.59	1.75	2.58	0.47

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Bacteria Application Results
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Kinetin 15mg/L	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	23	5.5	2.5	0.61
2	25	4.5	4.5	0.76
3	30	7	6.75	0.71
4	24	4.5	3.25	1.2
5	27	4.5	7.5	0.81
6	30	6	5.25	0.93
MEAN	26.50	5.33	4.96	0.84
STD. DEV.	3.02	1.03	1.95	0.21

Kinetin 20mg/L	PL. HEIGHT	I NODE LNGTH	LAT.BRNCH avg	DRY WGHT
1	23	3.5	3.5	0.57
2	23	4	4	0.59
3	19	3.5	2.5	0.7
4	27	5	2.25	0.81
5	18	2.5	2.5	0.57
6	25	4	3.5	0.44
MEAN	22.50	3.75	3.04	0.61
STD. DEV.	3.45	0.82	0.71	0.13

AG-Agrobacterium gluteans
RL-Rhizobium leguminosarum
CF-Cornybacterium fascians
PL.- Plant
I NODE- Internode
LAT. BRANCH- Lateral branching.
WGHT-Wieght
STD. DEV.- Standard Deviation