

Phytotoxicity and Efficacy of Fascination
(6-Benzyl Adenine + Gibberellic Acid)
for Enhanced Branching of
Field Marigold (*Calendula officinalis* 'Orange Mix')

By

Heiner Lieth, Director
Linda Dodge
Ron Lane
Jackie Fortunko

Project: Interregional Research Project #4
Project Number 23219A – November 25, 2004

Donors/Supporters:
CDFA Minor Crops Research Grant, Project 2: Enhancement of the Western
Region IR-4 Program to Address California Needs

UC Davis Environmental Horticulture IR4 Center
Department of Environmental Horticulture
University of California
One Shields Ave.
Davis, CA 95616
<http://envhort.ucdavis.edu/ir4>

PR.NO.:	23219A
TRIAL:	1
DATE:	11/25/04

IR-4 ORNAMENTAL DATA REPORTING FORM
(Please type or print)

1. INVESTIGATOR (Name, Address, Phone#):

Dr. Heiner Lieth
Department of Environmental Horticulture
University of California
One Shields Ave.
Davis, CA 95616
Ph 530-752-7198
FAX 530-752-1819
Email: jhlieth@ucdavis.edu

LOCATION OF TRIAL:

TRIAL TYPE:(check one) FIELD CONTAINER GREENHOUSE INTERIORSCAPE

2. PESTICIDE:

COMMON NAME: 6-Benzyl Adenine + Gibberellic Acid A₄ A₇ FORMULATION: 1.8% + 1.8% (w/w)

BATCH NO.: _____ PRODUCT: Fascination
EPA REG. NO. 27135 MFG: Valent

3. USE INFORMATION:

COMMON NAME: _____ SCIENTIFIC NAME: _____
PLANT: _____ Field Marigold Calendula officinalis 'Orange Mix'
REASON: enhanced branching of potted crops

SOIL TYPE OR TYPE OF POTTING MIX: UC Mix

% SAND 35 % SILT _____ % CLAY _____ % OM 65 % pH 6.5

SEEDING DATE 9/1/04 EMERGENCE DATE 9/11/04 TRANSPLANTING DATE 9/28/04

PLANT OR POT SPACING 6" ROW SPACING 6" POT SIZE 4-inch

PLOT SIZE 50 sq. ft. EXPERIMENTAL DESIGN randomized complete block NO.OF REPS 9
(3 blocks)

4. APPLICATION PARAMETERS:

TYPE OF APPLICATION: foliar

NO. OF APPLICATIONS 2 APPLICATION TYPE manual spray bottle
NOZZLE TYPE/SIZE _____ NOZZLE PRESSURE _____ DELIVERY RATE _____
CALIBRATION DATE(S) _____

5. APPLICATION SUMMARY:

APPL.DATE	RATES (ppm)*	GROWTH STAGE
10/14/04	0, 125 (0.5X), 250 (1X), 500 (2X)	3 weeks post-transplant
11/4/04	0, 125 (0.5X), 250 (1X), 500 (2X)	6 weeks post-transplant

*Be sure to provide units

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6. RAINFALL/IRRIGATION RECORDS:

INCLUDE RAINFALL/IRRIGATION INFORMATION (printouts, IR-4 forms, etc.)

Plants were watered as needed (at least once daily) with half-strength Hoagland's solution

7. OTHER PESTICIDES, FERTILIZER, LIME AND ADJUVANTS USED:

PRODUCT Tame AMOUNT 0.16 oz./gal DATE 10/26/04

PRODUCT Pedestal AMOUNT 0.08 oz./gal DATE 10/26/04

PRODUCT Azatin AMOUNT 5 oz./acre DATE 10/26/04

PRODUCT Pylon AMOUNT 1.5 mL/gal DATE 11/10/04

PRODUCT Conserve AMOUNT 6 mL/gal DATE 11/10/04

PRODUCT Conserve SC AMOUNT 0.22 oz./gal DATE 11/16/04

PRODUCT Avid AMOUNT 0.08 oz./gal DATE 11/16/04

8. NARRATIVE SUMMARY OF METHODS AND RESULTS: (Use more pages if needed)

Materials and Methods

Plant Material and Culture. Seeds of *Calendula officinalis* 'Orange Mix' (Pennington Seed Co.) were planted in a seedling tray on September 1, 2004 using a medium of 50% peat and 50% vermiculite. The seeds were allowed to germinate for two weeks in a mist bench with bottom heat of 75°F. The seedlings were transplanted to 4-inch pots containing UC Mix on September 28, 2004 and maintained in a greenhouse under natural day length for 3 weeks until the experiment began on October 14, 2004. For the first three weeks of the experiment, the plants were grown in a greenhouse under natural day length with day/night temperatures of 80°F/63°F (27°C/17°C) (Figure 1). For the last three weeks of the experiment, the plants were grown in a greenhouse under natural day length with day/night temperatures of 78°F/65°F (26°C/18°C) (Figure 2). The plants were watered as needed (at least once daily) during the 6-week experiment with half-strength Hoagland's solution. Applications of pesticides as part of a normal pest management program were made as needed (see No. 7 above).

Experimental Procedure. Thirty-six plants were randomly chosen and individually tagged for treatment with 0, 125 ppm (0.5X), 250 ppm (1X) or 500 ppm (2X) Fascination with 9 replicates per treatment. These dosages were prescribed in an IR4 Fascination protocol dated 6/04 (Appendix A). The plants received the first of two foliar spray applications of the designated treatments on October 14, 2004 using manual spray bottles to spray leaves to runoff. The second application was made 21 days later on November 4, 2004. The plants were arranged in a randomized complete block design with 3 blocks and 3 treatment replicates per block. Phytotoxicity and efficacy measurements were taken at day 0 (October 14, 2004), day 21 (November 4, 2004) and day 42 (November 25, 2004). Phytotoxicity evaluations were based on a numerical rating scale of 0 (no injury) to 10 (complete kill) (Table 1). Efficacy measurements consisted of overall plant height (cm) measured from the surface of the container medium to the top of the canopy and width (cm) reported as the average of two horizontal canopy measurements taken perpendicular to each other. In addition, the numbers of branches per plant and flowers per plant were counted.

For each observation a canopy volume index was calculated so as to be able to determine if canopy volume was affected by the application of Fascination. The calculation was made as $H*W_1*W_2$ for observations where two width measurements were available and as $H*W*W$ where only one width measurement was available. The usefulness of this index is based on the fact that many of the models for such a volume calculation are of the form $a*H*W_1*W_2$, where H is the height, W_1 and W_2 are two width measurements. The constant "a" depends on the assumption of the shape of the canopy. Since analyses of variance are scale-independent, the conclusion will thus be for the volume of the plant canopy.

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Statistical Analysis. The data were analyzed using Proc GLM of the Statistical Analysis System (SAS). The phytotoxicity and change in mean value from the starting plant height, width, volume index, branch number and flower number were analyzed for significant differences using t-tests.

Results

Phytotoxicity Index. The phytotoxicity index values of Calendula plants treated with Fascination ranged from 5 to 7 at week 3 and from 3 to 9 at week 6 (Appendix B). The increases in phytotoxicity index from week 0 ranged from 4.6 to 6.3 at week 3 and 4.1 to 7 at week 6 (Figure 3, Table 2). The phytotoxicity index of the control plants changed only as much as 1.3 by week 6. Even the 0.5X rate showed phytotoxicity index levels of 4.6 and 4.1 at 3 and 6 weeks, respectively. Plant damage symptoms included chlorosis and puckering of leaves leading to distorted growth (Figure 4). Symptoms occurred as early as three weeks after the first application of Fascination. By week 6, one third of the plants in the 2X treatment were severely stunted and wilting with reduced root masses (Figure 4).

Efficacy. At week 3, the control plants increased 7 cm in height which was significantly less than the 15.7, 18.1, and 14.7 cm increases in the 0.5X, 1X, and 2X Fascination treatments, respectively (Figures 3 and 5, Table 2, Appendix C). At week 6, this pattern changed with no significant differences in height increase between the control (30 cm taller than at the start) and the 1X treatment (35 cm taller than at the start), while 0.5X treated plants were 40 cm taller than at the beginning of the experiment and 2X treated plants were only 20 cm taller than at the beginning.

No differences in width increases were seen at week 3 (Figures 3 and 5, Table 2, Appendix C). By week 6, however, the plants in the 2X treatment increased only 5.5 cm in width, while the plants in the 1X treatment were 10 cm wider and the control plants were 18 cm wider.

Branch counts increased significantly more by week 3 in the Fascination treatments than in the control (Figure 3, Table 2, Appendix C). By week 6, the average branch count increases of the control (30.1) and of the 2X treatment (27.4) were not significantly different. The branch count increases in the 0.5X and 1X treatments (of 49.3 and 44.9, respectively), while not significantly different from each other, were different from the control and 2X treatment.

Discussion

Recommended greenhouse day/night temperatures for Calendula production are relatively cool (62°F/58°F or 17°C/14°C). Temperatures in this experiment were considerably higher: 80°F/63°F (27°C/17°C) for the first three weeks and 78°F/65°F (26°C/18°C) for the last three weeks. This may explain the excessive growth seen in the control plants and may have influenced the level of phytotoxicity seen in the plants treated with Fascination.

Phytotoxicity. The phytotoxicity of all Fascination treatments was substantial and so severe as to make the plants unmarketable. Thus we conclude that Fascination should not be used on Calendula at even the lowest rate.

Efficacy. Branch counts were affected by Fascination, resulting in significant increases in branching. Unfortunately the phytotoxicity is so severe that this effect cannot be exploited by growers. Thus this product should not be used on Calendula.

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9. GOOD RESEARCH PRACTICE STATEMENT:

I acknowledge that I have read and followed the IR-4 Research protocol and completed this trial following good agricultural practice, or reported any deviations (note any changes from authorized protocol in narrative).

SIGNATURE _____ DATE
PRINCIPAL INVESTIGATOR

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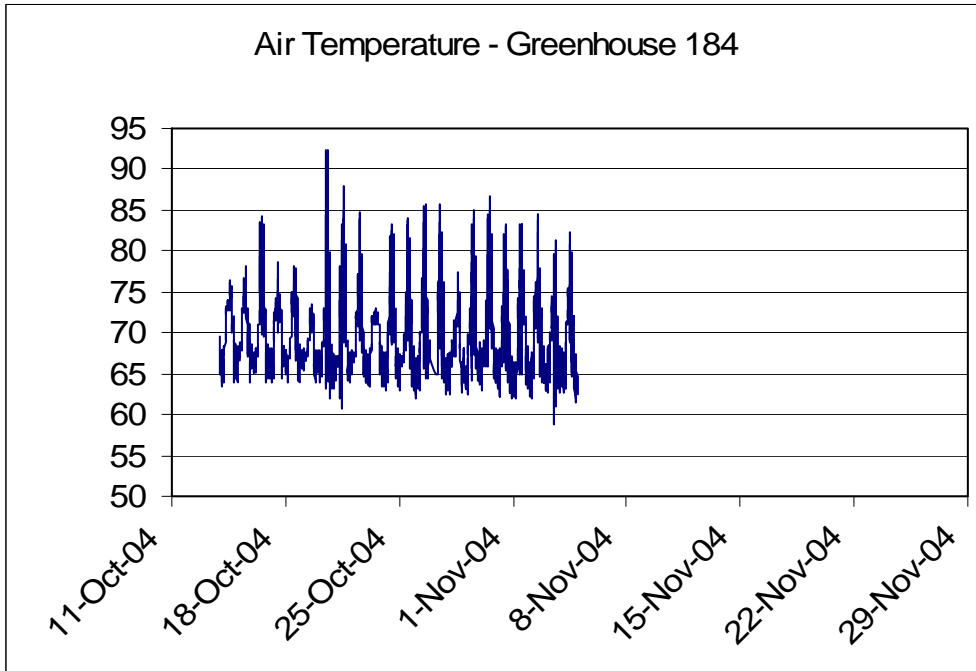


Figure 1. Greenhouse temperatures during the first three weeks of the experiment to evaluate the effects of Fascination on *Calendula officinalis* 'Orange Mix'.

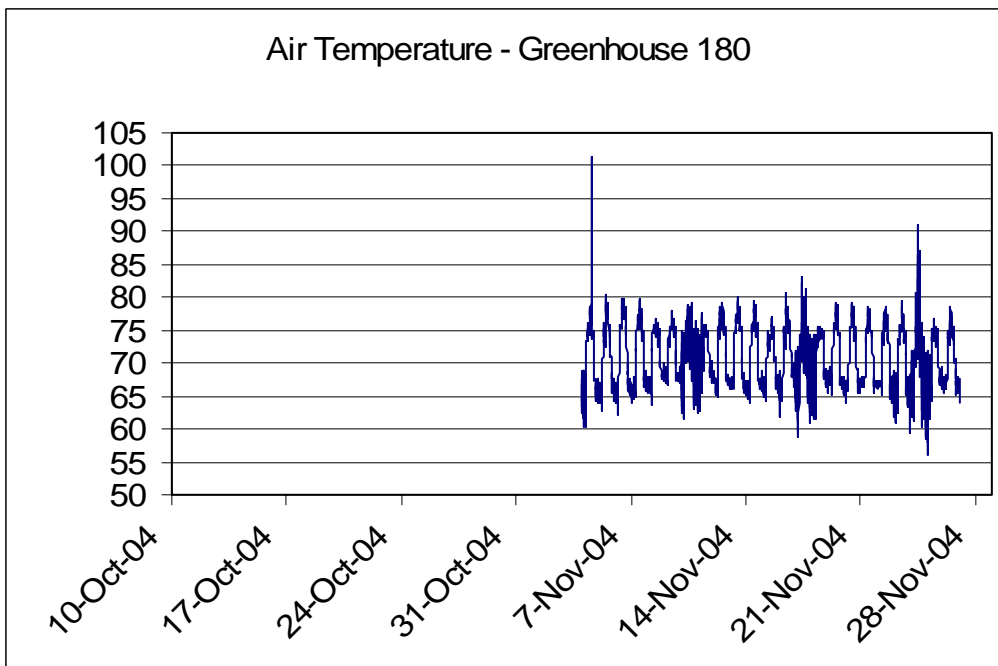


Figure 2. Greenhouse temperatures during the last three weeks of the experiment to evaluate the effects of Fascination on *Calendula officinalis* 'Orange Mix'.

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Table 1. Numerical plant damage rating scale used for phytotoxicity determinations.

Rating	Description of plant damage
0	No damage
1	No visible damage but unintended (non-permanent) impact
2	Slight leaf/tissue damage (curling leaves, necrosis, etc.)
3	Marginal chlorosis on some leaves (damage on up to 10% of plant)
4	10% – 20% of plant damaged
5	Significant damage to much of plant (30% - 40%)
6	40% – 60% of plant damaged
7	Chlorosis or necrosis on most of plant (60% - 70%)
8	Abscised leaves, branch dieback
9	Tissue severely damaged (80% - 100% of plant)
10	Complete kill

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Species: *Calendula* -- Material: Fascination

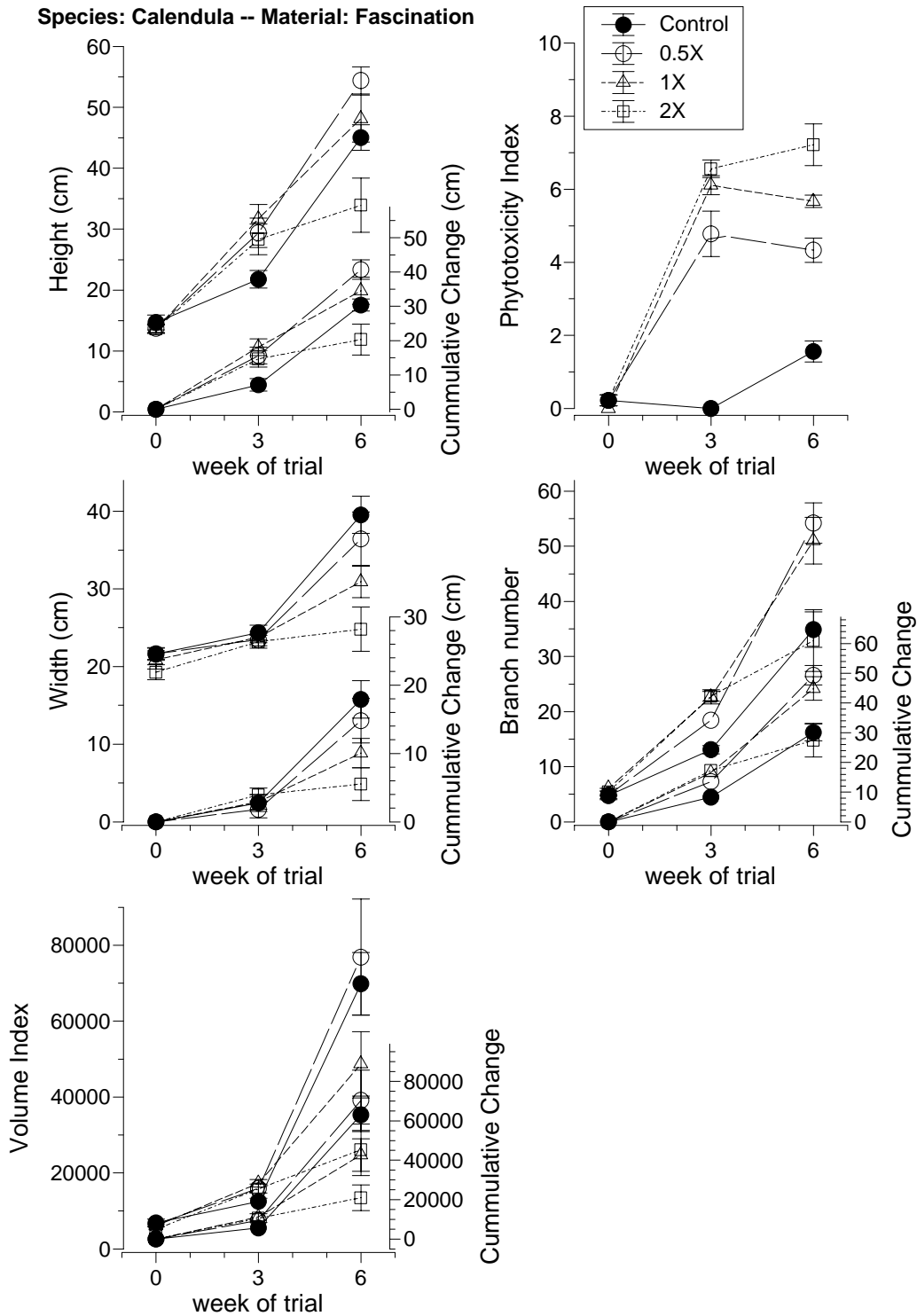


Figure 3. Summary of results for *Calendula officinalis* 'Orange Mix' treated with 0, 125, 250 or 500 ppm Fascination. Both raw data and cumulative changes over time are plotted for phytotoxicity index, plant height, plant width, branch number and volume index. SE bars shown. n=9.

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Table 2. Summary of results for *Calendula officinalis* 'Orange Mix' treated with 0, 125, 250 or 500 ppm Fascination. Cumulative changes over time are reported for phytotoxicity index, plant height, plant width, branch number, volume index and number of flowers per plant. Different letters within a column indicate significant differences between treatments ($P < 0.05$). "Yes"/"No" refer to significant treatment effects at the 5% level. Means \pm SE ($n=9$).

Fascination on Calendula				
Phytotoxicity Increase after:				
Treatment	3 weeks	yes	6 weeks	yes
0 ppm (Control)	-0.22 \pm 0.15	a	1.33 \pm 0.29	a
125 ppm (0.5X)	4.56 \pm 0.60	b	4.11 \pm 0.31	b
250 ppm (1X)	6.11 \pm 0.26	c	5.67 \pm 0.17	c
500 ppm (2X)	6.33 \pm 0.29	c	7.00 \pm 0.65	d
Height Increase after:				
Treatment	3 weeks	yes	6 weeks	yes
0 ppm (Control)	7.11 \pm 1.82	a	30.39 \pm 1.76	a
125 ppm (0.5X)	15.67 \pm 2.45	b	40.72 \pm 2.83	b
250 ppm (1X)	18.11 \pm 2.47	b	34.56 \pm 3.95	ab
500 ppm (2X)	14.72 \pm 2.39	b	20.33 \pm 4.50	c
Width Increase after:				
Treatment	3 weeks	no	6 weeks	yes
0 ppm (Control)	2.75 \pm 1.22	a	17.92 \pm 2.73	a
125 ppm (0.5X)	1.83 \pm 1.26	a	14.81 \pm 3.25	ab
250 ppm (1X)	2.92 \pm 1.10	a	10.06 \pm 2.15	bc
500 ppm (2X)	3.92 \pm 1.03	a	5.53 \pm 2.40	c
Relative Volume Index Increase after:				
Treatment	3 weeks	no	6 weeks	yes
0 ppm (Control)	5677.13 \pm 1302.37	a	63028.1 \pm 8537.69	a
125 ppm (0.5X)	9428.54 \pm 1403.66	ab	70381.3 \pm 15281.9	a
250 ppm (1X)	11207.5 \pm 828.81	b	42654.0 \pm 8245.75	ab
500 ppm (2X)	10585.6 \pm 2239.96	b	20904.1 \pm 6498.87	b
Branch number Increase after:				
Treatment	3 weeks	yes	6 weeks	yes
0 ppm (Control)	8.33 \pm 1.11	a	30.11 \pm 2.85	a
125 ppm (0.5X)	13.56 \pm 0.71	b	49.33 \pm 3.30	b
250 ppm (1X)	16.56 \pm 0.93	c	44.89 \pm 3.97	b
500 ppm (2X)	17.33 \pm 0.73	c	27.44 \pm 5.65	a
Total Flower+Bud number at:				
Treatment			6 weeks	yes
0 ppm (Control)			37.89 \pm 3.75	a
125 ppm (0.5X)			52.67 \pm 5.29	a
250 ppm (1X)			47.89 \pm 7.12	a
500 ppm (2X)			14.11 \pm 4.99	b

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Week 3



Week 6



Week 6

Figure 4. Plant damage symptoms seen on *Calendula officinalis* 'Orange Mix' treated with 0, 125, 250 or 500 ppm Fascination. Top: Control (left) and 500 ppm Fascination (right) at Week 3. Center: Plants treated with 250 ppm Fascination, 1X rate (left), and 500 ppm Fascination, 2X rate (right), at Week 6. Bottom: Distorted leaf growth (left) and reduced root mass (right) seen at Week 6 on plants treated with 500 ppm Fascination.

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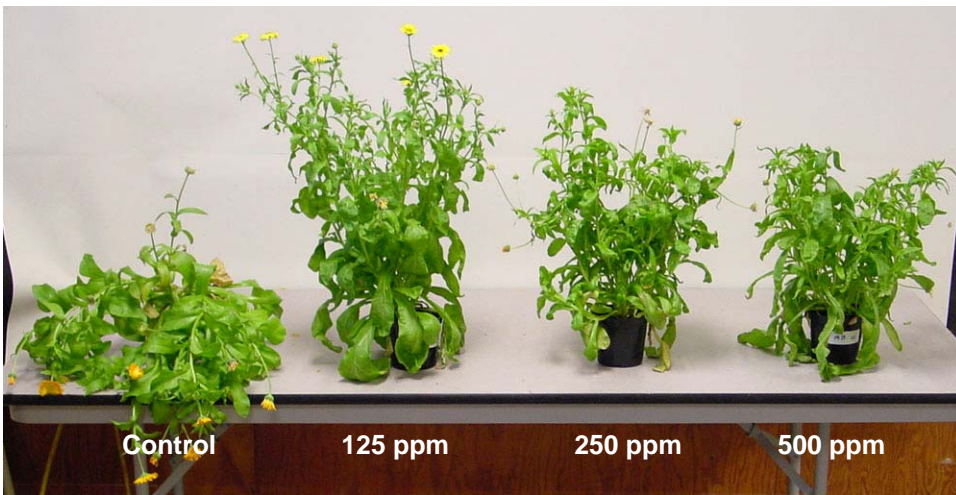
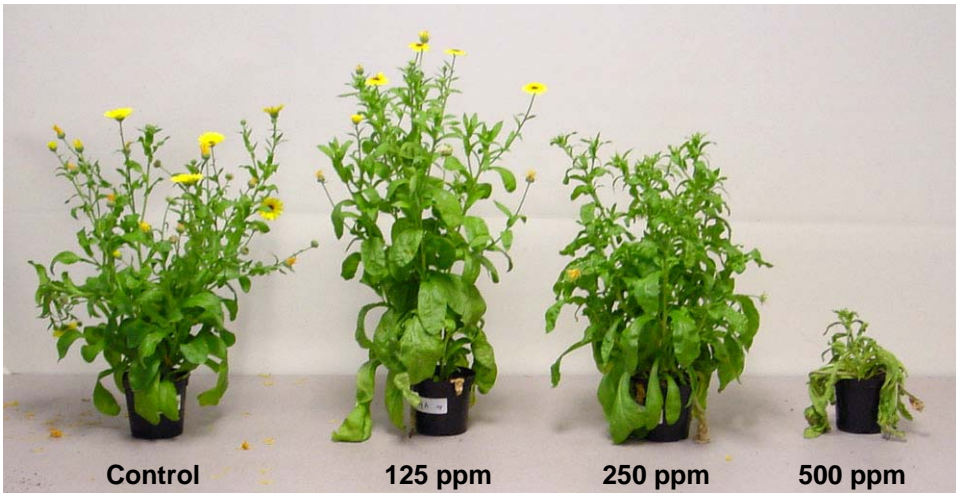


Figure 5. Representative plants of *Calendula officinalis* 'Orange Mix' 6 weeks after 2 foliar applications of 0, 125, 250 or 500 ppm Fascination.

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Appendix A:

GROWTH ENHANCEMENT OF ORNAMENTAL PLANTS WITH FOLIAR APPLICATIONS OF 6-BENZYL ADENINE + GIBBERELIC ACID (FASCINATION)

Date: 6/04

Ornamental Protocol Number: 670

General label directions: Refer to product label or Technical Bulletin.

Research program:

Pest(s)/Plants – Plants other than labeled species.

Pesticide (common name and trade name): Refer to treatment list shown below.

For label, material & if needed, spray surfactant contact:

Valent USA, Joe Chamberlin, 770-985-0303, icham@valent.com

Plot size (must be adequate to reflect actual use condition)

Replicates Minimum of 4 Treatment Units

Controls (untreated controls to be included in all experiments)

Application: **FASCINATION**

Dosages - 1/2x 125 ppm or 0.125 pt/5 gal.

1x 250 ppm or 0.25 pt/5 gal.

2x 500 ppm or 0.5 pt/5 gal.

Active Ingredient: 6-benzyl adenine + gibberellic acid (FASCINATION).

Volume - Minimum of 100 gal/A.

Timing - 2 Applications, 21 day spray interval. Record number of branches and length, then increased branching, increased extension and crop safety at 7, 14, 21 (then 2nd appl.), 28 and 42 DAT.

Reports:

Method of application: Treatments should be applied according to product label instructions. application equipment consistent with conventional commercial equipment. Report completely on experimental design and method of application. Report plant size height x width before treatment and throughout the experiment.

Weather – Maintain temperature and precipitation (including irrigation) data.

Soil type – Identify soil type used in experimental area.

Product – When submitting data, include EPA registration number of product used.

Efficacy – Data should include percent control as well as an indication that infestation was light, heavy, etc.

Record all application and evaluation dates.

Phytotoxicity – Record phytotoxicity data at all rates. Use a 0-10 scale. 0 = No Phytotoxicity 10 = complete kill.

Please direct questions to: Dr. Robert M. Herrick, IR-4 Project, 681 US Highway #1 South, North Brunswick, NJ Phone: (732) 932-9575, Ext. 629.

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Appendix B: PHYTOTOXICITY REPORT FORM

Calendula Data			Phyto	Phyto	Phyto
			Rate	Rate	Rate
Fascination					
Treatment	Block	Rep	10/14/2004	11/4/2004	11/25/2004
Control	A	1	0	0	2
Control	A	2	1	0	2
Control	A	3	0	0	0
Control	B	1	0	0	2
Control	B	2	0	0	2
Control	B	3	0	0	2
Control	C	1	0	0	0
Control	C	2	1	0	2
Control	C	3	0	0	2
		Mean	0.22	0.00	1.56
		Std Dev	0.44	0.00	0.88
125 ppm	A	1	0	6	5
125 ppm	A	2	0	5	3
125 ppm	A	3	0	5	3
125 ppm	B	1	0	6	5
125 ppm	B	2	0	5	5
125 ppm	B	3	1	6	5
125 ppm	C	1	0	0	3
125 ppm	C	2	0	5	5
125 ppm	C	3	1	5	5
		Mean	0.22	4.78	4.33
		Std Dev	0.44	1.86	1.00
250 ppm	A	1	0	7	6
250 ppm	A	2	0	6	6
250 ppm	A	3	0	6	6
250 ppm	B	1	0	7	6
250 ppm	B	2	0	7	6
250 ppm	B	3	0	6	6
250 ppm	C	1	0	5	5
250 ppm	C	2	0	6	5
250 ppm	C	3	0	5	5
		Mean	0.00	6.11	5.67
		Std Dev	0.00	0.78	0.50
500 ppm	A	1	0	7	9
500 ppm	A	2	0	7	9
500 ppm	A	3	1	6	6
500 ppm	B	1	1	7	6
500 ppm	B	2	0	7	6
500 ppm	B	3	0	7	9
500 ppm	C	1	0	6	5
500 ppm	C	2	0	5	9
500 ppm	C	3	0	7	6
		Mean	0.22	6.56	7.22
		Std Dev	0.44	0.73	1.72

NOTE: DEFINE MEASUREMENT OF PHYTOTOXICITY, OR INDEX OF INJURY (0=NO INJURY, 10=COMPLETE KILL) (See Table 1)

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Appendix C: EFFICACY REPORT FORM

Calendula Data											
			Plant	Plant	Plant	Plant	Plant	Plant			
			Height	Height	Height	Width	Width	Width	# Branches	# Branches	# Branches
Fascination			(cm)	(cm)	(cm)	(cm)	(cm)	(cm)			
Treatment	Block	Rep	10/14/04	11/4/04	11/25/04	10/14/04	11/4/04	11/25/04	10/14/04	11/4/04	11/25/04
Control	A	1	8.5	25	41	23.75	23.25	45.5	7	11	46
Control	A	2	15	15	41.5	22	27.5	37	4	12	19
Control	A	3	18	24.5	48.5	17	21.75	42	7	14	42
Control	B	1	12.5	24.5	49.5	21.25	21.5	34.75	6	10	35
Control	B	2	14.5	23.5	51	20	24	47.5	2	13	39
Control	B	3	22	24	53	26	21.75	34.5	7	13	42
Control	C	1	14	21	36	21.25	23.75	51	3	16	20
Control	C	2	14.5	24.5	48	21	26	32	6	17	39
Control	C	3	13	14	37	22.25	29.75	31.5	1	12	32
		Mean	14.67	21.78	45.06	21.61	24.36	39.53	4.78	13.11	34.89
		Std Dev	3.72	4.29	6.27	2.47	2.85	7.18	2.33	2.26	9.62
125 ppm	A	1	12.5	26.5	56	22.5	26	30	3	17	43
125 ppm	A	2	13.5	23.5	52	22.75	22.25	54	7	18	66
125 ppm	A	3	10	21	63	21	23	31	4	20	37
125 ppm	B	1	13.5	44.5	58	23.75	21.5	52	5	20	70
125 ppm	B	2	13	27.5	52.5	20	24.5	30.5	5	16	47
125 ppm	B	3	14.5	33.5	55	20	22.5	37	7	20	58
125 ppm	C	1	15.5	26.5	58.5	18.5	28	24.25	2	17	50
125 ppm	C	2	13	35.5	56	20.75	20.5	39.5	3	19	54
125 ppm	C	3	18	26	39	25.5	23	29.75	8	19	63
		Mean	13.72	29.39	54.44	21.64	23.47	36.44	4.89	18.44	54.22
		Std Dev	2.20	7.24	6.67	2.17	2.33	10.37	2.09	1.51	11.02
250 ppm	A	1	12.5	27.5	50	18	26	23	4	20	40
250 ppm	A	2	13	27	32	20.5	23.5	25.5	5	22	40
250 ppm	A	3	13.5	28	43	20.75	22.75	32.75	8	22	70
250 ppm	B	1	16	23	34	21.5	27	26	7	23	35
250 ppm	B	2	12	32	66	17.75	23	33	5	20	53
250 ppm	B	3	10.5	41	41.5	15.25	20	34	9	30	58
250 ppm	C	1	14	27.5	61.5	26.25	25.25	28.75	6	27	42
250 ppm	C	2	16.5	44	52.5	24.5	22.25	43.75	7	23	68
250 ppm	C	3	14.5	35.5	53	23.25	24.25	31.5	4	17	53
		Mean	13.61	31.72	48.17	20.86	23.78	30.92	6.11	22.67	51.00
		Std Dev	1.90	7.06	11.59	3.50	2.12	6.16	1.76	3.87	12.68
500 ppm	A	1	13.5	17	13	18.5	22.75	14	7	20	8
500 ppm	A	2	14	25.5	14.5	19	24	14	7	27	27
500 ppm	A	3	12	25	41	16.25	19.75	22	6	23	31
500 ppm	B	1	11	29.5	48	19.25	25.25	26.5	2	18	37
500 ppm	B	2	15	35	33	23.25	23.75	35	5	22	63
500 ppm	B	3	10.5	21	28.5	15.5	22.25	19	3	23	26
500 ppm	C	1	16.5	33	48	16.75	23.75	29	8	25	55
500 ppm	C	2	13.5	42	45.5	22.75	27.5	38.5	6	23	26
500 ppm	C	3	16.5	27	34	22.25	19.75	25.25	5	24	23
		Mean	13.61	28.33	33.94	19.28	23.19	24.81	5.44	22.78	32.89
		Std Dev	2.18	7.56	13.35	2.90	2.47	8.57	1.94	2.64	16.82