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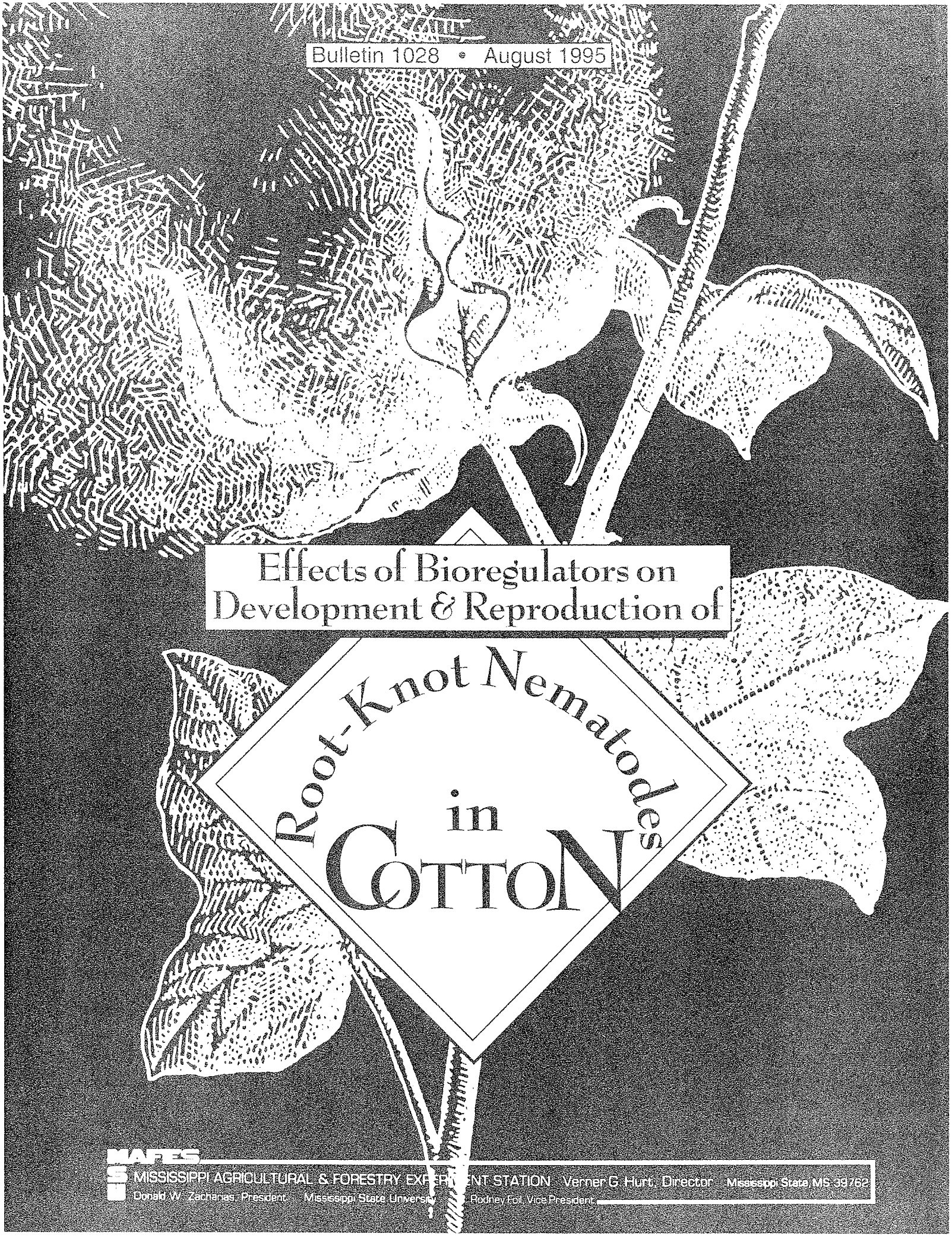

Effects of Bioregulators on Development & Reproduction of Root-Knot Nematodes in Cotton

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Abstract

The root-knot nematode (RKN) *Meloidogyne incognita* (Kofoid and White) Chitwood is a sedentary endoparasite that retards growth and development of cotton *Gossypium* spp. by attacking the root system, causing galling, stunting, and other adverse effects. Several resistant genotypes have been developed; however, transfer of the resistant genes to improved cultivars through conventional genetic techniques is costly and time-consuming. It was considered of interest to determine whether any of the plant growth hormones or related candidate bioregulators stated to have effects on plant roots or aerial parts could also control RKN growth and development. Of all the bioregulators tested, only 0.03-0.1% aqueous gibberellic acid (GA_3) solutions provided an intermediate resistance; i.e. total RKN eggs were reduced to 20% of the susceptible, inoculated control; whereas, RKN eggs were reduced to 3% of the susceptible cultivar in roots of the RKN-resistant inoculated control. In two of five tests, GA_3 significantly depressed root growth, however. On the other hand, endogenous gibberellic acid may have a more efficient role in imparting RKN resistance.

Introduction

The root-knot nematode (RKN) *Meloidogyne incognita* (Kofoid and White) Chitwood is a sedentary endoparasite that retards growth and development of cotton *Gossypium* spp. by attacking the root system, causing galling, stunting, and other adverse effects. Shepherd et al. (1988a,b) reported that the most RKN- resistant cultivars, Aub-634 and M-120 RNR (glanded) and 89-8275 (glandless) contained from 1,200 to 5,000 eggs per plant, whereas the susceptible lines Coker-201 (glanded), Aub-201 (glandless), and M-8 (glanded) contained from 6,000 to more than 100,000 eggs per plant at 40 days after inoculation.

Production of a large number of RKN eggs in susceptible roots in a relatively short time is associated with a tremendous amount of damage inflicted upon the young cotton seedlings by the nematode. As the galls increase in size, the root cortex surrounding the galls splits, exposing a relatively large area of the central cylinder (Mace et al., 1978). RKN also increases the incidence and severity of other soilborne diseases such as Fusarium wilt caused by *Fusarium oxysporum* Schlect f. sp. vasinfectum (Atk) Snyd and Hans (Bell, 1986).

The history of breeding for cotton (*Gossypium hirsutum* L.) cultivars with resistance to root-knot nematodes (*M. incognita* can be traced to the early nineteenth century (Ware, 1936). Since then, a series of resistant or tolerant cotton germplasms and breeding lines has been developed. However, recent research (Jenkins et al., 1993) indicates that cotton cultivars and most germplasm resources in the United States are susceptible to *M. incognita,* although cultivars vary considerably in their degree of susceptibility.

The development of nematode-resistant cultivars is time-consuming, limited by interspecific barriers between *Gossypium* spp. and by the difficulty in identifying homozygous resistant individuals in large, mostly susceptible progenies from crosses of resistant and susceptible parents. Biotechnology has provided a potential for efficient development of nematode-resistant plants that may be enhanced by the elucidation of mechanisms limiting nematode development, or by the identification of plants possessing nematode resistance. However, the application of this new technology for root-knot nematode resistance in cotton requires a sound understanding of the nematode biology and host-parasite interactions.

A somewhat associated development is the steadily increasing use of natural and synthetic bioregulators to improve or alter growing patterns and yield. In some instances, the bioregulator acts to modify plant gene expression, affecting levels of DNA, RNA, enzymes, and finally, their products such as proteins, carbohydrates, lipids, and allelochemicals. In the control of pests, the biosynthesis of allelochemicals by the plant, relatively steady state or event induced, can increase yield by limiting or even eliminating damage to the economically important tissues, typically the fruit.

The bioregulators kinetin and Burst® (a commercial preparation of naturally occurring cytokinins including zeatins), when applied as foliar sprays to cotton, increased the content of known allelochemicals and evidently also contributed to plant resistance to the tobacco budworm [*Heliothis virescens* (Fab.)] (Hedin and McCarty, 1994) because modest increases in yield were observed. The effects of the plant growth regulator mepiquat chloride [1,1-dimethylpiperidinium chloride (Pix®)] on cotton, including its allelochemicals, have been widely studied and have been summarized in a recent review (Hedin, 1990). Bud gossypol was increased, while flavonoids and tannins were slightly decreased. Yields tended to be decreased, but the use of Pix may still be advantageous because of enhanced maturity. Other bioregulators (BAS 109® , BAS 110® , and BAS 111®) increased gossypol, tannins, and flavonoids in cotton leaves (Hedin et al., 1988a, 1988b).The objective of this study was to immerse roots of susceptible and resistant cotton seedlings in aqueous solutions of several plant growth hormones or other compounds

stated to have effects on plant roots or aerial parts. It was hoped that the development of the RKN would be arrested at some stage and that some insights into the mechanisms of RKN development would be gained. The general criteria for the selection of the candidate compounds, information about their original development, and their reported mechanisms of action are summarized in two recent reviews of Hedin (1990) and Hedin and McCarty (1994).

Materials and Methods

Cotton Plant Sources

The host plants used in this experiment were the susceptible cotton cultivar ST-213 (S) and the resistant germplasm ST-213 RNR (a near isogenic line resulting from Auburn 634 x ST-213 with seven backcrosses).

Nematodes

The *Meloidogyne incognita* race 3 population used was originally isolated from cotton and was maintained on cotton in the greenhouse with an occasional generation on tomato (*Lycopersicum esculentum* L.). It was confirmed as race 3 by using the North Carolina State University differential host test (Myers, 1990; McPherson, 1993). Procedures used in this study were, unless otherwise indicated, those of Creech et al. (1995).

Eggs used for inoculation were removed from egg masses by shaking in 1.31% NaOCl for 4 minutes, followed by a thorough rinse with tap water, and then collected with a 25-micrometer opening mesh sieve.

Second-stage juveniles (J2's) for inoculation were obtained from eggs that were then placed on 25-micrometer opening mesh sieves in water and maintained at 28° ± 1 °C for 2 days. The hatched J2's passed through the sieve and were collected every 24 hours for 4 days. They were maintained in water at 4 °C until inoculation.

Bioregulators

The bioregulators and their sources were gibberellic acid (GA_3), indoleacetic acid, kinetin, urea, hydroxyurea, cysteine, salicylic acid, abscisic acid, chlorocholine chloride (CCC), ancymidol, N-acetyl-glucosamine (NAGA), and colchicine from Sigma Chemical Co., St. Louis, MO; Biochanin A, Fluka Chemical Corp., Ronkonkoma, NY; methyl jasmonate, Bedoukian Research Inc., Danbury, CT; PIX (mepiquat chloride), all-cis-8-(4-chlorophenyl)-3,4,8-triazatetracyclo-[4.3.1.$0^{2,5}0^{7,9}$] dec-3-ene (BAS-109), 1-phenoxy-5,5-dimethyl-3-[1,2,4-triazolyl-1]-pentan-4-ol (BAS-111), and N,N-dimethyl-piperidinium chloride (DMC) from BASF Aktiengesellschaft, Limburgerhof, Germany; and PG-IV, stated to contain the micronutrients Mg (1.0%), Cu (0.05%), Zn (0.05%), Fe (0.10%), B (0.02%), Mo (0.0005%), and Co (0.0005%), and the hormones indolebutyric acid (0.001%) and gibberellic acid (0.001%) from Microflo Co., Lakeland, FL. Given the preliminary nature of the tests, no attempt was made to determine the quantity that adhered to the root, the amounts absorbed, or metabolites formed.

Test Procedures

For tests 1-4 (see Tables 1-4), seedling roots were treated by soaking in bioregulator solutions for 0.5 minute. After the seedlings were transplanted into 10-cm clay pots filled with methyl bromide fumigated soil (Wickham sandy loam soil, a fine loamy, mixed thermic typic hapludult), each clay pot was inoculated with 5,000 eggs. Pots containing one plant each were maintained at 28 ± 2 °C in the greenhouse.

The experimental design was a randomized complete block with five replications of one pot each. Forty days after inoculation, seedlings were gently washed with tap water and the fresh shoot and root weights of each sample were measured. The whole root system was stained with phloxine B (Daykin and Hussey, 1985) for egg mass counting. Eggs were washed from egg masses as before. The eggs per egg mass, egg masses per root weight, and eggs per root weight were calculated.

Test 5 (see Table 5), was carried out as with Tests 1-4 except that each clay pot was inoculated with 1,000 J2's. The experiment design was a randomized complete block with 5 replications of 14 pots each. Individual pots in each replication were sampled at 2-day intervals beginning 2 days after inoculation through 14 days. At each sampling date, plants were removed from pots and soil was gently removed from the roots by washing with tap water. Root-knot nematodes were then stained using the acid fuschin technique (Hussey, 1990). The total number of nematodes per whole root system were counted under the microscope. Also, the shoot weight, root weight, and shoot height of each sample were measured over time (Tang et al., 1994).

Statistics

The measurements made included fresh shoot and root weights per plant, number of egg masses, number of eggs, and number of eggs per egg mass. The ratios of egg mass/root weight and eggs/root weight, and total nematodes per root system with each sampling day were calculated. The data were normalized to percentile values with regard to that obtained from susceptible, untreated inoculated plants. Statistical significance is indicated from ANOVA (DiIorio, 1991) and detected by LSD.

Results and Discussion

A series of tests was conducted to study the effects of candidate bioregulators in which seedling roots of susceptible (ST-213) plants were immersed. In the first test, the results of which are summarized in Table 1, shoot and root weights of RKN-susceptible cotton plants were significantly less than susceptible controls (suggesting phytotoxicity), particularly

Table 1. Effects of plant growth regulators on susceptible inoculated cotton plants at 40 Days. Percent of susceptible (ST-213) inoculated plants[a,b].

Test[c]	PGR Level, %	STWT	RTWT	MASS	EGGS	EG MS	MS RWT	EG RWT
S	0	100ABC (3.86)	100AB (4.40)	100BC (101.4)	100AB (71640)	100A (716.6)	100CD (24.4)	100ABC (17523)
GA_3	0.1	65.0E	57.0D	11.6C	10.1D	82.4AB	20.0E	16.9E
IAA	0.1	80.5BCDE	99.3AB	95.8BCD	76.8ABC	82.1AB	95.1CD	73.4ABCD
Kinetin	0.1	71.5CDE	60.6CD	50.8DE	41.7CD	81.0AB	82.5CD	62.9BCDE
PIX	0.1	94.0ABCD	85.5ABCD	116.3AB	102.7A	84.3AB	129.8BCD	112.3AB
NAGA	0.1	110.9A	113.4A	126.2ABC	103.5A	79.6AB	64.1BCD	84.4ABCD
Urea	0.1	76.5CDE	83.6ABCD	57.3B-G	40.7CD	78.5AB	60.3DE	41.4DE
Hydroxyurea	0.1	84.8A-E	83.0ABCD	158.9A	98.5AB	61.1B	193.2A	124.5A
Cysteine	0.1	108.5AB	109.0ABC	102.1BC	83.5ABC	78.4AB	90.6CD	72.4BCD
Salicylic Acid	0.1	66.2DE	74.4BCD	64.3BCDE	51.2BCD	65.3AB	70.5CDE	54.2CDE
Biochanin A	0.1	73.2CDE	71.9BCD	104.3BC	83.5ABC	69.5AB	161.9ABCD	94.6ABC

[a] Data expressed as a percentage of the susceptible, inoculated, untreated seedling, actual values in parentheses. Means within columns followed by different letters are different ($P \leq 0.05$, Fisher's LSD).

[b] STWT = Shoot Weight (fresh), RTWT = Root Weight (fresh), Mass = Egg Masses/Plant, Eggs = Number of Eggs/Plant, EG MS = Number of Eggs/Mass, MS RWT = Number of Egg Mass/Gm. Root, EG RWT = Number of Eggs/Gm. Root.

[c] See Materials and Methods for nomenclature of test compounds.

following treatment with GA_3, kinetin, salicylic acid, and Biochanin A. Nematode eggs were decreased most following treatment with GA_3 but also decreased significantly with kinetin and urea.

A second test (Table 2) included several compounds that had caused significant differences in plant growth and egg content in the first test. They were evaluated at two or more levels. Treatment of the data was the same as in Table 1. GA_3 reduced egg content significantly at treatment levels of 0.1, 0.03, and 0.01%, exhibiting an intermediate level of resistance in comparison with the inoculated, resistant cotton line. Root fresh weight was near normal at the 0.01% level. Kinetin and urea reduced egg content only marginally, and kinetin was somewhat phytotoxic. Salicylic acid reduced egg content but also was phytotoxic. N-acetyl glucosamine (NAGA) was inactive while BAS-109, although reducing egg content, was phytotoxic, causing sharp decreases in growth of the shoot and root system. The ST-213 resistant line exhibited greater shoot and root growth than the susceptible isoline, and the egg content was reduced to only about 6% of the susceptible line (Tang et al., 1994).

A third test (Table 3) included four plant growth hormones, a mixture of two of them, a commerial plant growth regulator formulation that included several micronutrients and the plant growth hormones IBA and GA_3, four synthetic gibberellic acid antagonists, three compounds (colchicine, salicylic

Table 2. Effect of PGR's on susceptible inoculated cotton plants at 40 days. Percent of susceptible (ST-213) inoculated plants[a,b].

Test[c]	PGR Level, %	STWT	RTWT	MASS	EGGS	EG MS	MS RWT	EG RWT
S	0	100AB (3.40)	100ABCD (3.56)	100AB (127.8)	100AB (43,740)	100AB (362.8)	100ABC (35.9)	100AB (12302)
R	0	107.4A	120.7A	5.8E	6.4E	105.4A	5.0F	5.5E
GA_3	0.1	79.9CD	91.9B-F	24.3DE	19.4E	73.9BCD	24.6EF	19.2E
GA_3	0.03	76.7D	87.2CDEF	25.4DE	15.1E	57.6D	29.0EF	19.1E
GA_3	0.01	82.2BCD	93.5BCDE	32.3DE	26.9E	81.9ABCD	33.7DEF	17.4DE
Kinetin	0.1	71.8D	76.1DEF	80.4AB	80.7ABC	93.3ABC	122.9A	128.2A
Kinetin	0.03	83.5BCD	86.5CDEF	70.3BC	63.2CD	85.6ABCD	77.1BCD	70.3BCD
NAGA	0.1	106.5A	105.7ABC	82.6AB	84.9ABC	100.6AB	80.0ABC	81.8
NAGA	0.03	102.6A	102.2ABC	100.6AB	100.8AB	97.5AB	98.5ABC	98.5AB
Urea	0.1	107.7A	113.9AB	90.7AB	82.4ABC	83.7ABCD	78.7ABC	69.9BCD
Urea	0.03	102.6A	104.8ABC	108.7A	109.5A	98.5AB	105.0ABC	105.0AB
Salicylic Acid	0.1	75.5D	66.7FG	44.4CD	34.6DE	67.1CD	68.4CDE	50.7CDE
Salicylic Acid	0.03	97.4ABC	92.4BCDE	97.0AB	78.6BC	77.1ABCD	105.7ABC	85.5ABC
BAS-109	0.1	43.3D	43.1G	8.2E	6.1E	59.8D	18.4F	13.4E
BAS-109	0.03	71.7D	68.6EFG	74.0BC	73.8BC	94.5ABC	116.7AB	114.2AB

[a] Data expressed as a percentage of the susceptible, inoculated, untreated seedling, actual values in parentheses. Means within columns followed by different letters are different ($P \leq 0.05$, Fisher's LSD).

[b] STWT = Shoot Weight (fresh), RTWT = Root Weight (fresh), Mass = Egg Masses/Plant, Eggs = Number of Eggs/Plant, EG MS = Number of Eggs/Mass, MS RWT = Number of Egg Mass/Gm. Root, EG RWT = Number of Eggs/Gm. Root.

[c] See Materials and Methods for nomenclature of test compounds.

Table 3. Effect of PGR's on susceptible inoculated cotton plants at 40 days. Percent of susceptible (ST-213) inoculated plants[a,b]

Test[c]	PGR Level, %	STWT	RTWT	MASS	EGGS	EG MS	MS RWT	EG RWT
S	0	100BC (4.35)	100ABCD (7.86)	100ABC (122.8)	100AB (100662)	100ABC (836.0)	100B (15.6)	100BC (12829)
R	0	122.2AB	118.0AB	3.7E	3.0G	68.8BCD	3.4E	2.8G
GA_3	0.1	119.9ABC	101.5ABCD	28.3DE	21.5FG	76.0ABCD	29.3CDE	22.1FG
IAA	0.1	111.0ABC	109.1ABCD	86.8BC	79.3BC	95.2ABC	89.5BCD	80.8BCD
ABA	0.1	121.2ABC	115.2AB	86.4BC	72.3BC	85.0ABCD	82.9BCD	68.9B-F
GA_3 + IAA	0.1,0.1	94.6BC	77.3CDEF	63.0CD	42.8DEF	66.5CD	87.9BCD	59.4B-F
Kinetin	0.1	124.3AE	109.9ABC	62.2CD	62.8CD	102.8AB	60.6BCDE	60.2B-F
CCC	0.1	105.4ABC	90.3BCDE	37.6DE	41.0DEF	108.6A	44.4BCDE	47.7D-G
DMC	0.1	135.0A	134.6A	38.1DE	38.7DEF	103.7AB	28.7DE	28.6EFG
PG-IV	0.1	119.3ABC	117.8AB	90.0BC	81.4BC	87.6ABCD	84.1BCD	77.0BCDE
BAS-111	0.1	88.7C	41.0F	41.5DE	40.1DEF	95.8ABC	111.6B	107.5B
Ancymidol	0.1	96.3BC	71.7DEF	133.5A	113.8A	88.9ABCD	200.1A	166.5A
Colchicine	0.1	117.2ABC	107.8ABCD	64.3CD	58.5CDE	89.0ABCD	64.6BCDE	56.3C-F
Salicylic Acid	0.1	115.9ABC	123.8AB	124.6AB	121.2A	95.3ABC	100.3B	97.7BC
Methyl Jasmonate	0.1	92.1BC	54.8EF	63.2CD	34.1EF	58.3D	99.3BC	54.7C-F

[a] Data expressed as a percentage of the susceptible, inoculated, untreated seedling, actual values in parentheses. Means within columns followed by different letters are different ($P \leq 0.05$, Fisher's LSD).

[b] STWT = Shoot Weight (fresh), RTWT = Root Weight (fresh), Mass = Egg Masses/Plant, Eggs = Number of Eggs/Plant, EG MS = Number of Eggs/Mass, MS RWT = Number of Egg Mass/Gm. Root, EG RWT = Number of Eggs/Gm. Root.

[c] See Materials and Methods for nomenclature of test compounds.

acid, and methyl jasmonate) reported to invoke various gene responses, and finally the resistant line.

GA_3 again sharply decreased egg content with no reduction of shoot or root weight. Two GA antagonists, CCC and DMC, were not measurably phytotoxic, and they decreased egg content to 40% of the susceptible line. Two other GA antagonists, BAS-111 and ancymidol, were phytotoxic, but only BAS-111 reduced egg content. Colchicine, which was not phytotoxic, reduced egg content by 41%.

None of the other plant growth hormones (IAA, ABA, kinetin, and PG-IV) were phytotoxic, but they decreased egg content only by about 30-40%. A 1:1 mixture of GA_3 and IAA (0.1% each) was moderately phytotoxic, but was fairly effective in that it reduced egg content by about 60%. Salicylic acid was neither phytotoxic nor effective in reducing egg content. Methyl jasmonate reduced egg content by about 65%, but it was phytotoxic. The R isoline promoted greater growth than the S line and reduced egg content to about 3% of that on the S line.

A fourth test was conducted to determine whether those bioregulators that elicited intermediate decreases in egg content with minimal phytotoxicity (Table 3) would prove more effective at higher concentrations without exhibiting increased phytotoxicity. Unfortunately, on increases of the concentrations from 0.1 to 0.2%, CCC, DMC, and BAS-111 did not further depress the egg content (Table 4). The phytotoxicity was not increased with CCC, and not substantially increased with DMC. On the other hand, the increase of BAS-111 to

Table 4. Effect of bioregulators on susceptible inoculated cotton plants at 40 days. Percent of susceptible (ST-213) inoculated Plants[a,b]

Test[c]	PGR Level, %	STWT	RTWT	MASS	EGGS	EG MS	MS RWT	EG RWT
S	—	100ABC (9.2)	100BC (19.2)	100A (106.8)	100A (94592)	100A (883.5)	100A (5.6)	100AB (4925.7)
R	—	117.3A	120.3A	1.8EF	1.2E	49.4B	1.7E	1.0E
GA_3	0.1	97.8ABC	84.3CDE	22.0D	19.6DE	88.4A	26.7DE	23.8DE
CCC	0.1	102.1ABC	121.8A	68.9B	70.8B	102.0A	57.1CD	59.6BCD
CCC	0.2	103.2ABC	113.0AB	67.4B	64.7BC	97.0A	58.9CD	57.8CD
DMC	0.1	100.0ABC	110.9AB	68.9B	64.6BC	92.9A	64.2BC	61.0BCD
DMC	0.2	82.6C	82.2DE	77.5B	78.9AB	102.2A	94.6AB	99.0AB
BAS-111	0.1	86.9BC	79.1E	83.5AB	73.1B	87.7A	110.7A	94.5ABC
BAS-111	0.2	39.1D	15.1F	20.4DF	17.3E	80.1A	123.2A	111.6A
S NOT Inoc	—	106.5AB	110.4AB	0.0F	0.0E	0.0C	0.0E	0.0E

[a] Data expressed as a percentage of the susceptible, inoculated, untreated seedling, actual values in parentheses. Means within columns followed by different letters are different ($P < 0.05$, Fisher's LSD).

[b] STWT = Shoot Weight (fresh), RTWT = Root Weight (fresh), Mass = Egg Masses/Plant, Eggs = Number of Eggs/Plant, EG MS = Number of Eggs/Mass, MS RWT = Number of Egg Mass/Gm. Root, EG RWT = Number of Eggs/Gm. Root.

[c] See Materials and Methods for nomenclature of test compounds.

0.2% elicited a strong phytotoxicity. GA at 0.1% continued to depress egg content by about 80%, and elicited a modest decrease in root weight (16%).

In a fifth test (Table 5), the number of nematodes at 14 days, the shoot height and weight, and the root weight at intervals of from 2-14 days were recorded from S and R inoculated plants, half of which were treated by immersion in 0.1% GA_3. Four days after inoculation, the total numbers of nematodes were not significantly different in untreated and GA_3-treated susceptible plants. After 8 DAI, there were no evident differences of nematodes in resistant plants, with and without GA_3 treatment . The egg counts were low, most of the increase occurring later between 14 and 40 days as in previous tests. An elongation of GA_3-treated plants was observed.

Even though the GA_3-treated plants were 70-80% taller at 14 days, root weights were less than 50% of that on the untreated plants. Evidently, the less extensive root systems with fewer side roots of the GA_3-treated plants provided a decreased opportunity for nematode development.

From these tests, it was hoped that one or more of the candidate compounds, given their various reported physiological activities, might prevent establishment of nematode infestation in roots of susceptible plants while permitting normal root and shoot development. GA_3 was the most effective agent for decreasing nematode development, but this decrease was at the expense of root development. Particularly in tests one and four (Tables 1 and 4), GA_3 treatment reduced proliferation of new root tissues. J2 nematodes have been shown to preferentially penetrate new germinated root tips (Tang et al., 1994). At two decreased concentrations (0.03 and 0.01%, Table 2) root development was not appreciably impaired.

Two GA antagonists, CCC and DMC, decreased egg development by 60% while root development was near normal (Table 3), but the decreased egg content of a third (BAS-111) was at the expense of root development which was only 40% of normal. Colchicine, though obviously not suitable for field control of RKN, did not deter plant development while decreasing the egg content by 40% (Table 3). The plant growth hormones IAA, ABA, and kinetin mildly suppressed egg numbers while not suppressing plant growth.

This work suggests that further screening may identify an agent that could suppress egg numbers to less than 20% of the susceptible line while not suppressing root development. Given the continuing development of RKN resistant isolines, the use of an in-furrow agent does not seem a likely control agent. However, this work suggests that endogenous gibberellic acid in the plant may have some role in imparting RKN resistance.

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Table 5. Effect of GA_3 on growth and infestation of nematode inoculated cotton plants[a].

Test	GA_3 level, %	Days after Inoculation 2	4	6	8	10	12	14	% of S Control at Day 14
					No. of Nematodes (Root)				
S	0	6.6A	77.8A	158.2A	122.0A	156.2A	161.4A	176.6A	100
S	0.1	10.8A	19.0B	51.0B	36.8B	53.2B	59.4B	47.4B	26.7
R	0	8.6A	81.4A	129.8A	109.8A	24.6C	24.8B	24.2B	13.7
R	0.1	9.8A	23.8B	39.6B	44.2B	19.4C	22.4B	20.0B	11.3
					Shoot Height, cm				
S	0	—	—	7.5D	8.5B	9.2B	10.5B	12.6B	100
S	0.1	—	—	12.7A	15.2A	16.8A	20.5A	23.4A	185.7
R	0	—	—	8.5C	9.2B	9.9B	12.0B	14.3B	113.4
R	0.1	—	—	11.6B	14.4A	16.3A	20.8A	24.1A	191.2
					Shoot Weight (g)				
S	0	0.80B	—	1.02B	—	1.25A	—	3.17A	100
S	0.1	0.91A	—	1.29A	—	1.56A	—	3.04A	95.8
R	0	0.79B	—	0.81C	—	1.50A	—	3.58A	112.9
R	0.1	0.93A	—	1.14AB	—	1.31A	—	2.92A	92.1
					Root Weight (g)				
S	0	0.85A	—	1.44A	—	1.75AB	—	3.05A	100
S	0.1	0.85A	—	0.98B	—	1.26BC	—	1.35B	44.2
R	0	0.89A	—	0.99B	—	2.16A	—	3.27A	107.2
R	0.1	0.64B	—	0.65C	—	1.10C	—	1.34B	43.9

[a] Means within columns for each trait followed by different letters are different ($P \leq 0.05$ Fisher's LSD).

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