# Influence of Cytokinin on In Vitro Screening of Peaches for Resistance to Nematodes

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**ABSTRACT**

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Growth on two levels of the cytokinin 6-benzylamino purine (BA) influenced the initial in vitro response of peach rootstock Nemaguard plantlets to *Meloidogyne incognita* but did not influence the response of the scion cultivar Jerseyqueen. The high level (2 mg/L) of BA resulted in an initial loss of resistance to *M. incognita* as evident with initial galling; however, the nematodes failed to develop to maturity and the plantlets recovered. This initial susceptible response was not observed in vitro after growth on the lower level (0.2 mg/L) of BA. Plantlets derived from Nemaguard and Jerseyqueen propagated and rooted in vitro were susceptible to *Pratylenchus penetrans* and differed significantly from controls in root weight, stem height, and numbers of leaves. This research suggests that in vitro screening of self-rooted peach lines may facilitate early detection of resistance to *M. incognita* regardless of the cytokinin influence.

Tissue culture and genetic engineering technologies can produce and propagate novel agriculturally useful plants (5). These techniques are currently being used to increase the availability of new germ plasm in peaches as well as to propagate known scion cultivars (6). In both cases, these self-rooted peach plantlets need to be evaluated for resistance to soilborne pathogens and pests.

Screening for plant resistance to nematodes on root explant cultures has been successful for soybeans (7). Palys and Meredith (12) used dual cultures of grape, either from rooted shoot tips or excised roots, to screen for tolerance of these cultivars to the nematode *Pratylenchus vulnus*. Other studies on determining resistance to nematodes by plants from tissue culture have led to conflicting results. Fassuliotis and Bhatt (3) demonstrated that resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood was lost in tomato leaf disk cultures, but *Solanum sisymbriifolium* regenerated from callus retained resistance to *M. incognita* but not to *M. javanica*. They proposed that the loss of resistance was caused by hormonal additives to the support media. Ammati et al (1) showed that tomato plants adventitiously reproduced from cotyledons remained resistant to root-knot nematodes in tissue culture. Therefore, hormonal additives need to be reconsidered to establish a protocol for in vitro screening for resistance to nematodes on peaches.

Screening for disease resistance with tissue culture techniques can be a problem if the tissues either do not express disease resistance in vitro (5) or lose disease resistance during the tissue culture process (3). Furthermore, cytokinins have been implicated as a possible factor in resistance of peach rootstocks to nematodes (1,9). Therefore, it is questionable whether peach tissue can be used in screening for nematode resistance, because these compounds are major components of tissue culture media.

The purpose of this study was to determine the feasibility of screening peach in vitro for resistance to *M. incognita* and *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven, both known pathogens of peach (2,10), and to determine the effects of exogenous cytokinins on resistance.

## MATERIALS AND METHODS

**Peach culture.** Peach (*Prunus persica* (L.) Batsch) rootstock Nemaguard, resistant to most root-knot nematodes (15), and *P. persica* scion cultivar

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**Table 1.** Number of *Prunus persica* cv. Nemaguard (N) and Jerseyqueen (JQ) plantlets inoculated with each nematode species per replicate and total number of controls per treatment

| Nematode | Cultivar | BA[a] | Rep 1 | Rep 2 | Rep 3 | Total | Controls |
|---|---|---|---|---|---|---|---|
| *Meloidogyne incognita* | N | H | 5 | 6 | 10 | 21 | 6 |
| *M. incognita* | N | L | 8 | 12 | ··· | 20 | 4 |
| *M. incognita* | JQ | H | 7 | 4 | 6 | 17 | 6 |
| *M. incognita* | JQ | L | 3 | 5 | ··· | 8 | 4 |
| *Pratylenchus penetrans* | N | H | 10 | 8 | 5 | 23 | 6 |
| *P. penetrans* | N | L | 5 | 5 | ··· | 10 | 4 |
| *P. penetrans* | JQ | H | 6 | 10 | ··· | 16 | 4 |
| *P. penetrans* | JQ | L | 7 | 7 | ··· | 14 | 4 |

[a]BA = 6-benzylamino purine; H = high level (2 mg/L) and L = low level (0.2 mg/L).

**Table 2.** Reactions of peach plantlets grown in vitro on low (0.2 mg/L) or high (2.0 mg/L) BA levels of cytokinin 5 wk postinfestation with *Meloidogyne incognita*[y]

| Plants examined | BA (mg/L) | Leaf count | | Shoot height (cm) | | Dry root weight (g) | |
|---|---|---|---|---|---|---|---|
| | | *M. incognita* | Control | *M. incognita* | Control | *M. incognita* | Control |
| Nemaguard | 2.0 | 14.4 a[z] | 15.3 a | 2.0 a | 2.1 a | 0.029 a | 0.280 a |
| | 0.2 | 14.2 a | 16.8 a | 2.1 a | 3.2 a | 0.028 a | 0.320 a |
| Jerseyqueen | 2.0 | 3.8 a | 12.5 b | 0.9 a | 1.9 b | 0.029 a | 0.030 a |
| | 0.2 | 9.5 a | 11.5 b | 1.9 a | 1.9 b | 0.039 b | 0.028 a |

[y] Values are the means of at least two replicates with a minimum of three plants per replicate for inoculated and two plants per replicate for uninoculated plants.
[z] Different letters represent significance in each pair according to Student's $t$ test ($P < 0.01$).

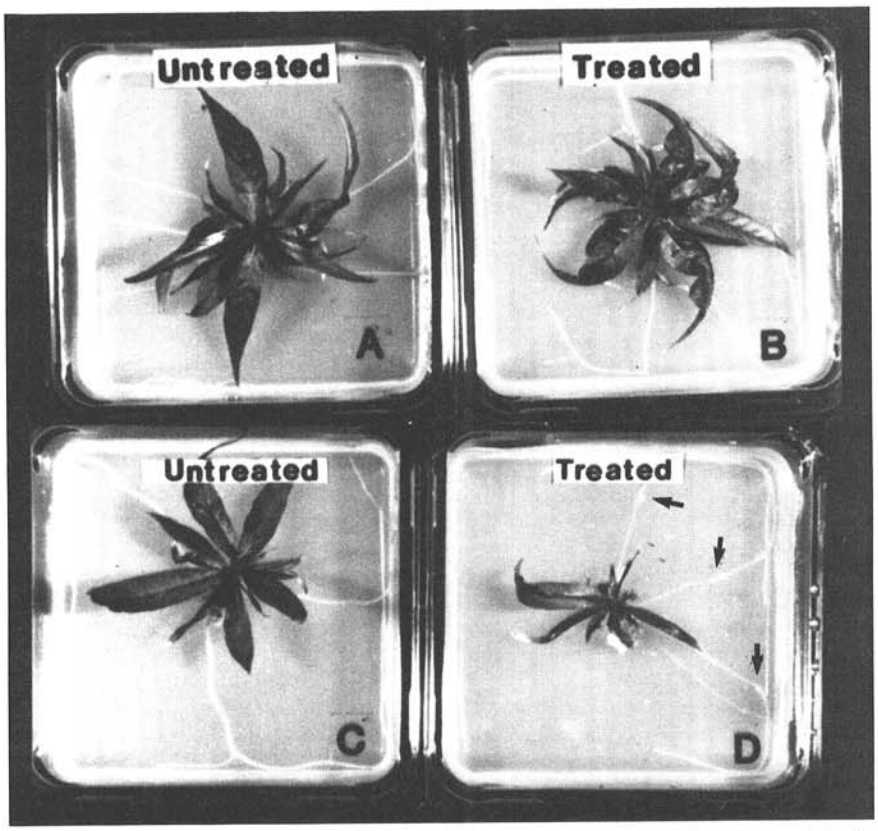


**Fig. 1.** Five-week-old peach rootstock Nemaguard cultured in vitro on 6-benzylamino purine at **(A and B)** 0.2 mg/L or **(C and D)** 2 mg/L, then rooted, transferred to one-half-strength MS medium (11), and either left untreated or treated with five egg masses each of *Meloidogyne incognita*.

Jerseyqueen were propagated on Murashige and Skoog (MS) medium (11) supplemented with 2% sucrose, and 0.6% Phytagar (Grand Island Biological Co., Grand Island, NY), and 0.4 mg of thiamine HCl, 100 mg of *myo*-inositol, 0.05 mg of nicotinic acid, 0.05 pyridoxine HCl, 0.01 *p*-aminobenzoic acid, 0.01 indolebutyric acid, and either 0.2 or 2 mg of 6-benzylamino purine (BA) per liter. All tissue culture media were adjusted to pH 5.6–5.8 and autoclaved at 1.4 kg/cm$^2$ for 15–20 min. Unless noted otherwise, all cultures were maintained at 26 C with a 16-hr photoperiod of about 40 $\mu$E m$^{-2}$s$^{-1}$ irradiance provided by cool-white fluorescent lamps. Shoots were rooted in the dark on one-half-strength MS medium supplemented as described and 0.53 mg/L 1-naphthaleneacetic acid. For nematode inoculations, rooted plantlets were transferred to one-fourth-strength MS medium without growth regulators and supplemented with 2% sucrose and 1% Phytagar.

**Nematode root explant cultures.** Nematodes were obtained from stock cultures maintained on sterile root explants (14) on B-5 medium of Gamborg et al (4). *M. incognita* was maintained on excised roots of *Lycopersicon esculentum* Mill. cv. Rutgers. *Pratylenchus penetrans* was maintained on *Zea mays* L. cv. Iowa Chief.

**Inoculation of plantlets.** Plantlets about 2 cm high were inoculated aseptically in vitro with either five egg masses of *M. incognita* or about 100 mixed life stages of *P. penetrans*. The number of treatments varied per replicate depending on the number of plantlets derived from the multiplication media. Each combination of nematode, plantlet, and either high or low BA was replicated either two or three times (Table 1). Two plantlets per replicate were used as controls.

All plantlets were incubated in a growth chamber at 28 C in the light. The following data were recorded after 5 wk: leaf counts, shoot height, and fresh and dry root weights. The number of galls with egg masses was determined for *M. incognita*. Only fresh root weights were taken for *P. penetrans*. Nematodes were recovered from the roots of the *P. penetrans*-infested plantlets as described by Rebois and Huettel (13).

**Analysis of data.** Data were analyzed using paired Student's $t$ tests.

## RESULTS

*M. incognita* on either the high or low level of BA failed to complete its life cycle on Nemaguard. There was no significant difference among treatments ($P < 0.01$) in the number of leaves, shoot height, or dry root weight (Table 2) except between the low-BA dry root weights. Nematodes penetrated the roots of Nemaguard plantlets on the high level of BA and some initial galling was observed (Fig. 1D); however, the nematodes failed to develop to maturity. All infected plantlets (Fig. 1B,D) recovered and were not significantly different in growth compared with the controls (Fig. 1A,C) after 5 wk.

On the Jerseyqueen plantlets infested with *M. incognita*, both levels of cytokinins for most observed variables were significantly different from the controls (Table 2). Nematode-infested plantlets were stunted and in some cases had become completely senescent. Heavy galling with viable egg masses was observed on the infested (Fig. 2B,D) but not in the control plantlets (Fig. 2A,C). Viability of the nematodes was determined by the live second-stage juveniles found in the agar and in some of the egg masses. There were fewer leaves and a reduction in shoot height of Jerseyqueen on the high levels of BA compared with the controls. The dry weights of the roots of susceptible plantlets were equal to or higher than those of the controls, because

the heavily galled roots with nematodes weighed as much or more than the controls.

Both Nemaguard (Fig. 3) and Jerseyqueen plantlets were susceptible to *P. penetrans*. There were significant differences in leaf count, shoot height, and dry root weight ($P < 0.01$) in infested plantlets on both cytokinin levels compared with the controls (Table 3). In roots that were stained and macerated, the number of nematodes recovered indicated as much as a 10-fold increase over the initial inoculation level after 5 wk.

## DISCUSSION

Growth substances appear to alter host-nematode relationships (1,16,17). For this reason, it must be demonstrated that resistance and susceptibility to plant-parasitic nematodes are clearly distinguishable in vitro before in vitro screening systems for resistance can be used.

In this study, susceptibility and resistance to *M. incognita* was easily recognizable in self-rooted plantlets propagated in vitro. Resistance was only slightly affected by high cytokinin levels in the multiplication media. The nematodes penetrated and caused slight initial galling on the Nemaguard plantlets; however, they failed to mature. No living nematodes were recovered from these small galls when they were teased out of the roots. The juvenile stages were completely clear internally and appeared to have become senescent soon after penetration. Cytokinin-induced cell elongation could have facilitated penetration of the nematodes, but other internal factors such as nutrition may have prevented development from continuing.

Resistance to *P. penetrans* was not observed in either Nemaguard or Jerseyqueen, but no resistance in peaches is known for this nematode. The susceptible response to this nematode did not appear to be affected by cytokinin levels either. Tolerance has been reported to occur in six clonal selections of peach (8). Therefore, screening procedures should include this nematode species to determine degrees of tolerance that could be introduced into new germ plasm.

These results suggest that screening peaches in vitro is a feasible approach for early detection of resistance to *M. incognita* and possibly to determine degrees of tolerance to *P. penetrans* in plants produced either through tissue culture or genetic engineering technologies. Cytokinins appear to play a role in resistance of peaches to root-knot nematodes (1,9). Consequently, tissue culture and/or genetic engineering technologies aimed at lowering cytokinin levels in peach roots could result in the development of new germ plasm with a higher degree of resistance.

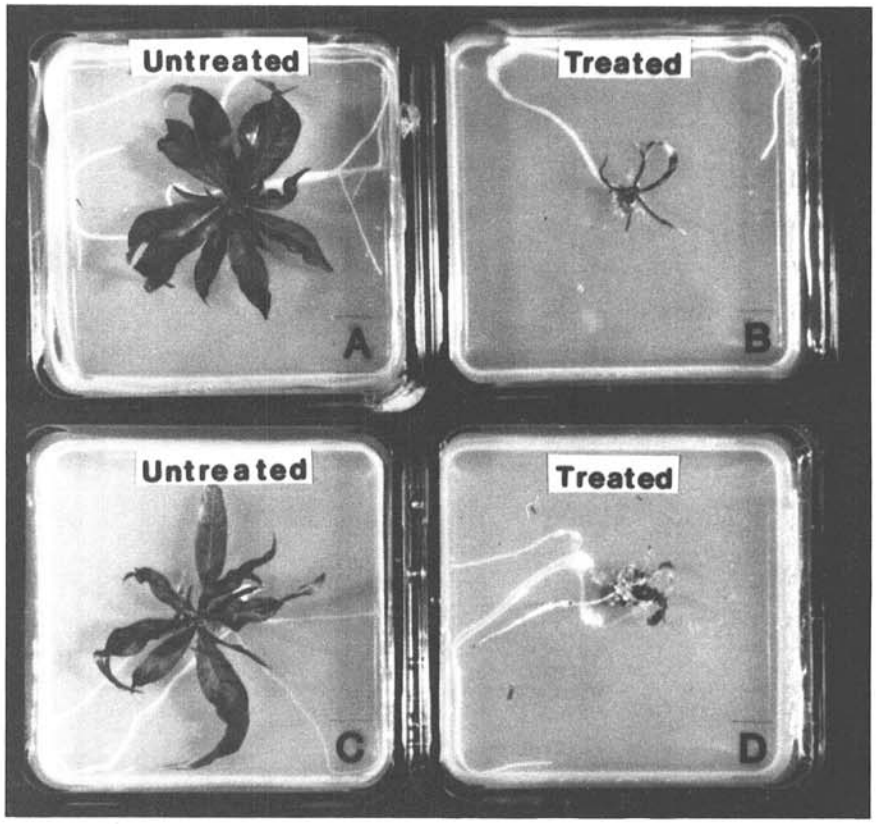


**Fig. 2.** Five-week-old peach scion cultivar Jerseyqueen cultured in vitro on 6-benzylamino purine at **(A and B)** 0.2 mg/L or **(C and D)** 2 mg/L, then rooted, transferred to one-half-strength MS medium (11), and either left untreated or treated with five egg masses each of *Meloidogyne incognita*.

**Table 3.** Effects of *Pratylenchus penetrans* (P) as compared to noninfested controls (C) on peaches propagated in vitro on two levels of cytokinins (0.2 or 2.0 mg/L BA) after 5 wk postinfestation[y]

| | 2.0 mg/L | | 0.2 mg/L | |
|---|---|---|---|---|
| **Plants examined** | **P** | **C** | **P** | **C** |
| Nemaguard | | | | |
| Leaf count | 5.80 a[z] | 17.30 b | 4.90 a | 18.20 b |
| Wet roots (g) | 0.05 a | 0.28 b | 0.38 a | 0.78 b |
| Shoot height (cm) | 0.99 a | 2.30 b | 1.50 a | 4.40 b |
| Jerseyqueen | | | | |
| Leaf count | 4.50 a | 12.30 b | 11.21 a | 20.50 b |
| Wet roots (g) | 0.10 a | 0.34 b | 0.35 a | 0.50 b |
| Shoot height (c) | 1.00 a | 1.60 b | 1.70 a | 2.30 b |

[y] Values are the means of at least two replicates with a minimum of five plants per replicate for inoculated plants and two plants per replicate for uninoculated plants.
[z] Different letters represent significance in each pair according to Student's *t* test ($P < 0.01$).

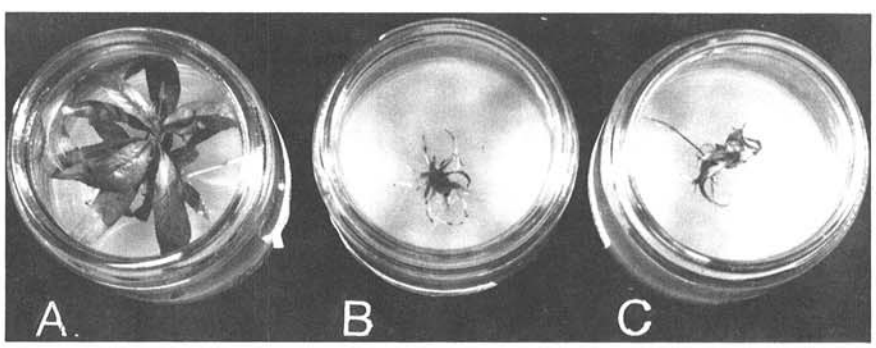


**Fig. 3.** Peach rootstock Nemaguard: **(A)** uninfested control; **(A and B)** rootstock after a 5-wk exposure to *Pratylenchus penetrans* on 6-benzylamino purine at **(B)** 0.2 mg/L or **(C)** 2 mg/L. Similar results were observed on Jerseyqueen (not pictured).

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