Abscisic Acid and Ethylene Increase in *Heterodera avenae*-infected Tolerant or Intolerant Oat Cultivars[1]

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Abstract: The relationship between root stunting caused by the cereal cyst nematode and levels of two root growth inhibiting hormones, abscisic acid and ethylene, was investigated in aseptically cultured root segments and in intact roots of two oat cultivars differing in tolerance to the nematode. Cultured root segments of oat cultivars New Zealand Cape (tolerant) and Sual (intolerant) were inoculated with sterilized *Heterodera avenae* second-stage juveniles. Suppressed growth of root axes and emerged laterals following nematode penetration corresponded to an increase in abscisic acid and ethylene in roots of both intolerant and tolerant cultivars. When the experiment was repeated on intact root systems, nematodes retarded root growth of Sual more than New Zealand Cape despite an increase in ABA and ethylene in both cultivars. Abscisic acid and (or) ethylene may be involved in growth inhibition of *H. avenae*-infected roots but appear to play no direct role in determining tolerance.

Key words: abscisic acid, *Avena sativa*, axenic culture, cereal tryst nematode, ethylene, *Heterodera avenae*, nematode, oat, plant hormone, root elongation, root explant, tolerance.

Impairment of root extension, commonly reported in plants infected with the cereal cyst nematode (*Heterodera avenae* Woll.) (18), is a cause of suppression of grain yield (12,21). Growth of nematode-infected roots is less impaired in plants that are tolerant to *H. avenae*; i.e., plants that yield well in spite of infection (24).

Abscisic acid (ABA) and ethylene, two naturally occurring plant growth regulators, inhibit root extension at physiological concentrations (4,8,17,25). This investigation was undertaken to determine whether root stunting of oat (*Avena sativa* L.) caused by *H. avenae* is due to localized changes in levels of ABA and (or) ethylene following nematode invasion and whether differences in the severity of growth impairment are related to differences in levels of these two hormones.

MATERIALS AND METHODS

Two oat cultivars, New Zealand Cape (NZC) which is tolerant and resistant and

Sual which is intolerant and resistant to *H. avenae* (3,23), were used for experimentation.

ABA and ethylene in agar-cultured inoculated root segments: Seeds were germinated on agar. The terminal 2 cm of seminal roots (3–4 cm long) were excised and transferred to 9-cm-d petri dishes containing a modified nutrient agar medium (14). Root segments were incubated in the dark at 22 C for 10 days. Segments (2 cm long) were cut from the main root and cultured for an additional 10 days on the same medium. From these cultures 2-cm root tips were excised and used as experimental material.

Ten 2-cm-long root apices were transferred, tip downward, to agar slants composed of 50 ml nutrient medium (14) with 1% Bacto Agar (Difco Laboratories, Detroit, MI) in 125-ml Erlenmeyer flasks. Flasks were stoppered with nonabsorbent cotton and aluminum foil and incubated at 22 C in the dark for 24 hours. Apices were then covered with 0.15 g autoclaved sand (grain size < 150 μm) and subsequently inoculated with surface-sterilized second-stage *H. avenae* juveniles (J2) applied at initial population densities (Pi) of 50 and 200 J2 per root tip. At 3-day intervals for 9 days, root apices were monitored for ethylene production and ABA content.

Ethylene measurements, replicated six

times on batches of 10 apices, were taken 24 hours after cotton plugs were replaced with rubber seals. Gas samples were drawn from each flask with a 1-ml syringe for injection into a gas chromatograph. Ethylene concentrations in the gases were measured by flame ionization gas chromatography on a glass column silanized and packed with Porapak Q (100–120 mesh; Waters Assoc., Milford, MA). Temperatures of the injector, column, and detector were 100, 40, and 100 C, respectively.

Measurements of ABA were replicated three or four times on batches of 10 apices each, which were frozen in liquid N_2 and freeze dried. Free ABA was detected using electron capture gas chromatography following extraction and purification from samples (7). A silanized 0-17 Gas Chrom Q (Applied Science Laboratories, State College, PA) glass column was used; operating temperatures of the injector, column, and detector were 210, 180, and 245 C, respectively. The minimal detectable amount of ABA was 3 pg, and the standard curve was linear between 0 and 1 ng. Losses during ABA extraction and purification were checked by adding 20 ng to the homogenizing medium before macerating. Recovery rates were as low as 45% in some cases but generally were about 75–80%. Corrections were made in calculations to account for losses.

Measurements were also made on the length of the main axis and first-order lateral roots, the number of unemerged and emerged root laterals, and the number of juveniles on roots stained in 0.1% cotton blue in lactophenol (22).

ABA and ethylene in roots of intact plants: Approximately 40 hours after germination, 15-mm-long coleoptiles were directed through 2-mm holes drilled in 2.4-cm rubber seals. The seals with seedlings attached were fitted onto open-ended plastic tubes (2.4 cm i.d. × 110 cm) lined with plastic bags of similar dimensions filled with steam-sterilized sandy loam with no fertilizer amendment. Seedlings received 10 ml half-strength Hoagland's nutrient solution (11) at planting. Plants were grown in a

cabinet at 20 C day and 15 C night with a 16-hour photoperiod. Total irradiance of combined high pressure sodium and incandescent bulbs was 590 $\mu E \cdot m^2 \cdot s^{-1}$. One day after planting, surface-sterilized J2 were applied to the surface of the soil at Pi of 500 and 1,500 J2 per plant in 1-ml aliquots through slits made in the rubber seals.

Every 3 days during the following 15 days, the root systems of nine intact plants were rinsed under a gentle stream of water to remove soil. The roots, with rubber seal still attached, were inserted into dark glass specimen tubes (2.35 cm i.d. × 9.6 cm) containing agar slants with the following nutrient concentrations (mM): KNO_3, 200; $CaNO_3$, 200; $MgSO_4$, 100; KH_2PO_4, 50; micronutrients, ¼ strength Hoagland's solution and Fe NaEDTA, 0.5 mg·1^{-1} as Fe. After inserting the rubber seal tightly, tubes were returned to the growth cabinet and placed at a 45° angle to facilitate contact between the roots and the agar. Ethylene samples were taken from the test tube with a 1-ml syringe at three 18-hour intervals. Rates of ethylene production were estimated from the mean value of three readings. After each sampling, fresh air (sterilized by passing through concentrated H_2SO_4, concentrated NaOH, sterile water, and two cotton filters) was introduced into the specimen tube through a syringe after first removing an equivalent amount of air. After ethylene sampling, three plants were set aside for measurement of root infection, length, and number, and six plants were used for ABA determinations after roots were snap frozen in liquid nitrogen and freeze dried.

Experiments were arranged in a randomized block design. Data were subjected to an analysis of variance and Fisher's test of least significant differences (LSD).

RESULTS

ABA and ethylene in agar-cultured inoculated root segments: The number of J2 in cultured root tips of NZC and Sual at similar Pi did not differ (Table 1). Nematode presence resulted in shortened root length and fewer primary lateral roots on infected
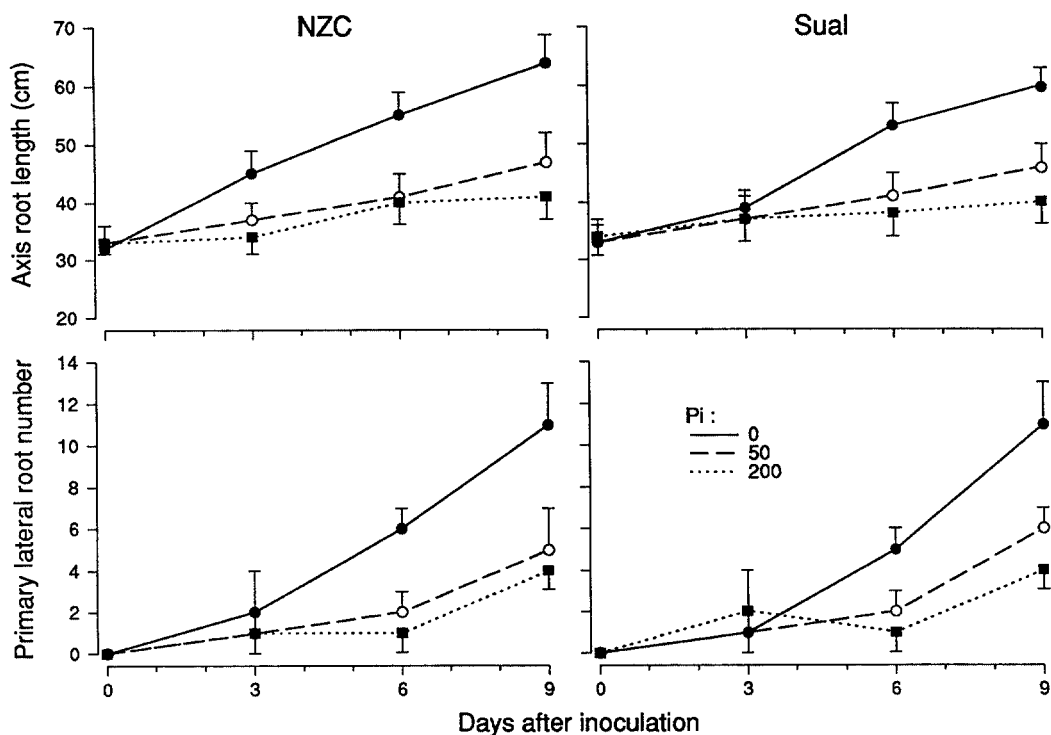
FIG. 1. Axis root length and number of laterals emerged from the root axes of cultured root segments of oat cultivars NZC and Sual following inoculation with 0 (●), 50 (O), or 200 (■) *Heterodera avenae* J2. Bars represent ± SE.

root segments (Fig. 1), with no differences between cultivars. Nematodes had no effect on the number of unemerged lateral root primordia (Table 1), although there tended to be fewer primordia on infected

TABLE 1. Effect of initial population density (Pi) on numbers of *Heterodera avenae* juveniles in root segments and on numbers of unemerged root primordia in root segments of two oat cultivars 3 to 10 days after inoculation.

Pi	H. avenae juveniles		Unemerged root primordia		
	3	9	3	6	10
New Zealand Cape					
0			7 a	17 b	29 b
50	4 a	5 ab	5 a	16 b	21 ab
200	9 b	7 b	5 a	13 ab	23 ab
Sual					
0			10 b	18 b	23 ab
50	4 a	4 a	7 a	16 b	24 b
200	7 ab	8 b	8 ab	13 ab	17 ab

Values are means of four replicates. Means within columns followed by different letters indicate differences ($P = 0.05$) based on Fisher's (protected) LSD test.

roots. Levels of ABA and ethylene were highest in infected roots after 6 and 3 days, respectively, (Fig. 2) before declining, with no differences between cultivars.

ABA and ethylene in roots of intact plants: The number of J2 in the roots increased with time and Pi (Table 2) but did not differ between Sual and NZC on the three sample dates.

Seminal root lengths of Sual and NZC were suppressed with increasing Pi, but only Sual was affected ($P = 0.05$) at the lowest Pi (Fig. 3). Total lateral root length and number were suppressed by Pi, with effects increasing with time (Fig. 4). Lateral root length of Sual was reduced ($P = 0.05$) at Pi = 500, whereas NZC was affected only at the highest Pi. Lateral root number declined in both cultivars at both Pi. Infection increased ABA concentration and ethylene production initially (Fig. 5), but differences in these measurements between infected and control plants de-
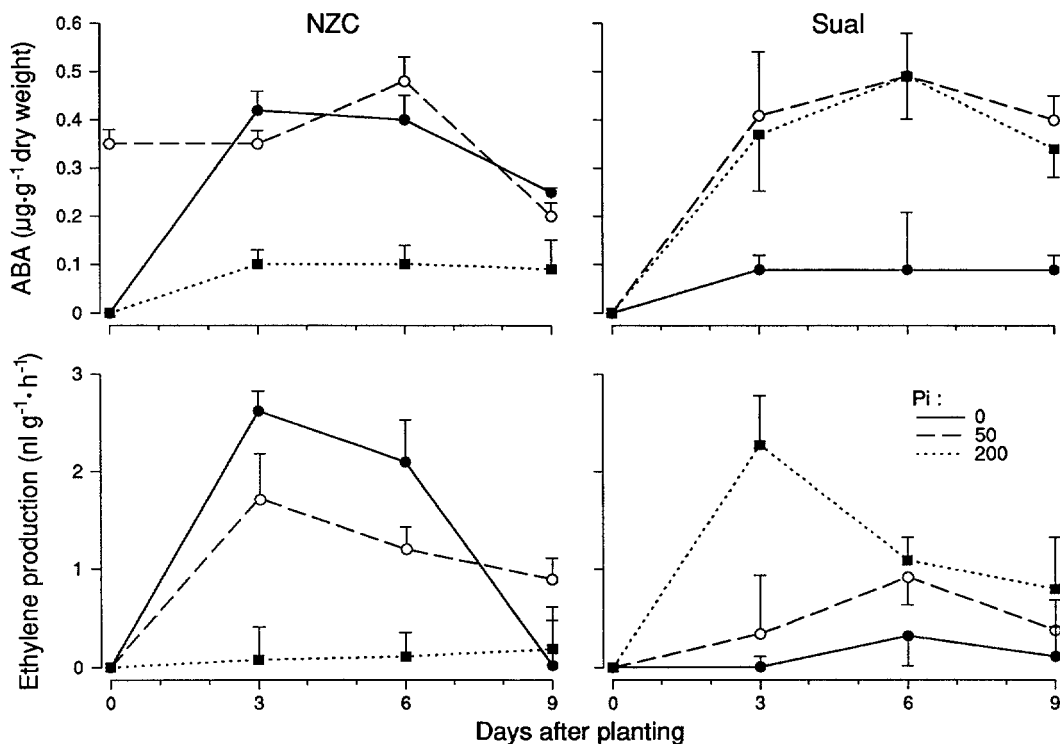
FIG. 2. Levels of abscisic acid (ABA) in and ethylene produced by cultured root segments of oat cultivars NZC and Sual following inoculation with 0 (●), 50 (○), or 200 (■) *Heterodera avenae* J2. Bars represent ± SE.

creased with time and did not occur between cultivars.

DISCUSSION

Inoculation of oat root segments and intact oat root systems with *H. avenae* J2 resulted in increased levels of ABA and ethylene as well as suppressed root growth of cultivars Sual and NZC. Symptoms of *H.*

TABLE 2. Effect of initial nematode population density (Pi) on number of *Heterodera avenae* juveniles in root systems of two oat cultivars 2, 8, and 14 days after inoculation.

Pi	2	8	14	LSD†
New Zealand Cape				
500	31 a	74 a	93 a	31
1,500	81 b	201 b	241 b	49
Sual				
500	46 a	86 a	102 a	29
1,500	72 b	218 b	267 b	44

Values are means of six replicates. Means within columns followed by different letters indicate differences ($P = 0.05$) based on Fisher's (protected) LSD test.
† LSD ($P = 0.05$) for means in the same row.

avenae infection included inhibition of lateral root emergence and impeded extension of root axes and laterals. These symptoms are typical of the effects on root growth of exogenously applied ethylene and ABA in the concentration range measured (1,2,4,5,13,25). The results therefore support the proposal that growth impairment of infected roots arises from increased root ABA and (or) ethylene. Ethylene and ABA concentrations measured in this study agreed closely with those in studies where nematodes were not used. The concentration of endogenous ABA in root tissue may vary from 0.4 μg·g^{-1} dry weight (DW) in decapitated pea plants (5) to about 1.1 μg/g^{-1} DW in root cultures on agar (10) and to as high as 2.2 μg/g^{-1} DW on sycamore roots (6). Ethylene production by intact unstressed seedlings of a range of plant species may vary from 2 to 6.5 nl·g^{-1}·h^{-1} (13). Elevated ethylene production has also been detected in tomato plants infected with *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (9).
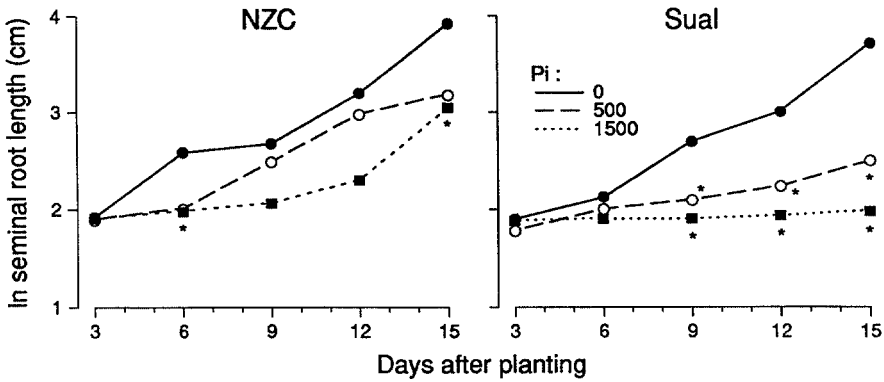
FIG. 3. Seminal root length from intact plants of NZC and Sual following inoculation with 0 (●), 500 (○), or 1,500 (■) *Heterodera avenae* J2. * Different from uninoculated control plants (*F*-test; $P = 0.05$).

Root segment growth of both Sual and NZC was affected by inoculation with *H. avenae* J2. Cultured root segments of NZC and Sual responded in a similar way to exogenously applied ABA and ethylene. Yet, in the intact plant, root elongation of Sual was more severely affected by *H. avenae* inoculation than was that of NZC, even though hormone levels in the two cultivars following inoculation did not differ. A previous study (24) also found root growth of Sual to be more impaired by infection than NZC. These results suggest that differences in tolerance between Sual and NZC are not due to differences in the levels of ABA or ethylene induced by *H. avenae*. Differences in tolerance could arise if root sensitivity to either of the two hormones was greater in Sual than in NZC.
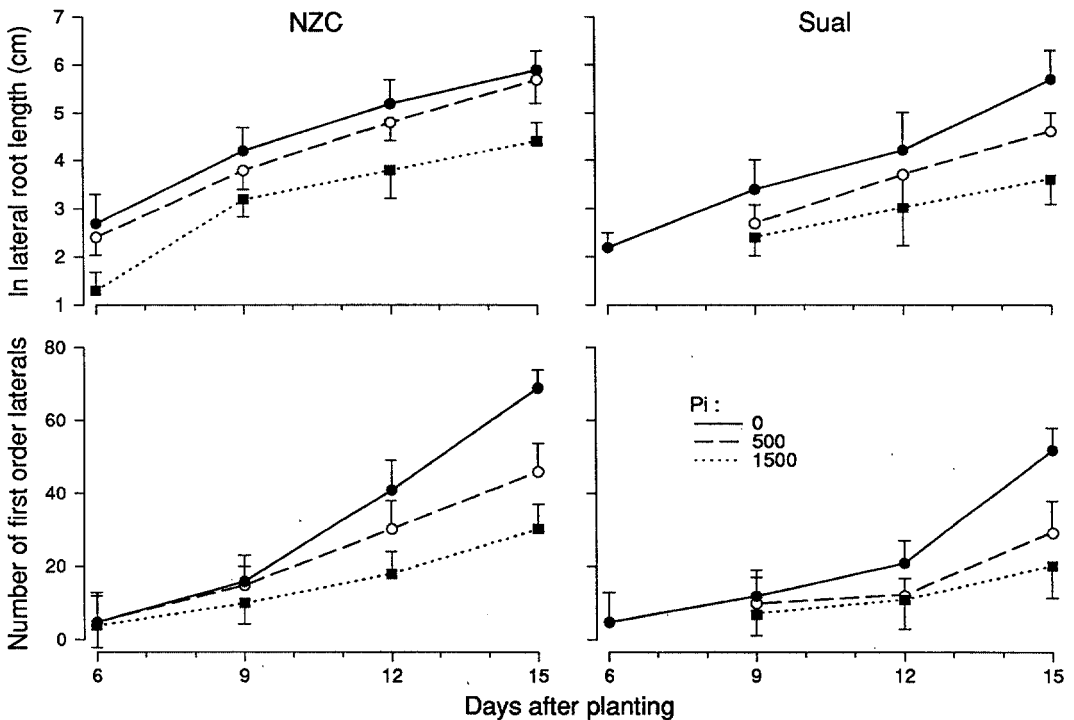
The detection of a differential growth

FIG. 4. Length and number of first order lateral roots emerged from seminal root axes of NZC and Sual following inoculation with 0 (●), 500 (○), or 1500 (■) *Heterodera avenae* J2. Bars represent ± SE.
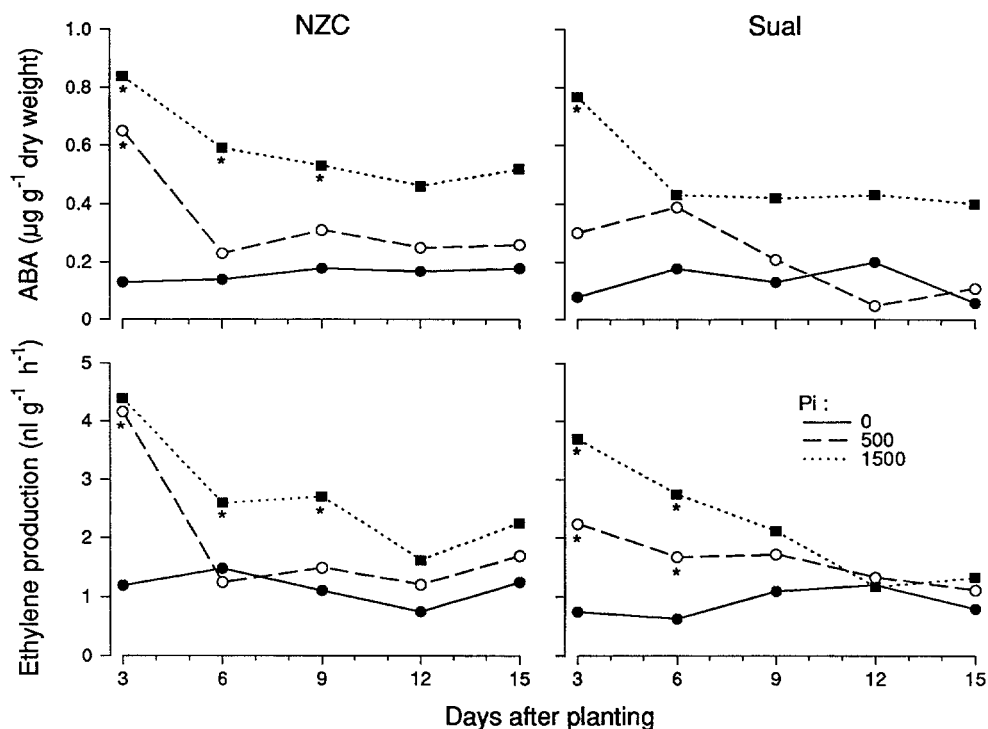
FIG. 5. Levels of abscisic acid (ABA) and ethylene produced in roots of intact plants of NZC and Sual following inoculation with 0 (●), 500 (○), or 1500 (■) *Heterodera avenae* J2. * Different from uninoculated control plants (*F*-test; $P = 0.05$).

response to *H. avenae* between cultivars in roots of intact plants but not in cultured root segments could also be a nutritional artefact of the experiment. Intact plants depend on a linear transport of nutrients which can be interrupted or competed for, whereas cultured roots are bathed in nutrients and are unlikely to be influenced by nutritional competition. A factor of shoot origin may also be involved in the response of roots to *H. avenae*. This factor could be hormonal; e.g., auxin is produced in the shoot and transported acropetally to the root tip (15,16). If so, then the infection process may result in loss of the ability of Sual to utilize or produce the hormone or to signal hormonal production. Other evidence supporting an hypothesis for hormonal involvement in the phenomenon of tolerance is scant. Plants lacking the capacity for ethylene production, e.g., the diageotropica mutant of tomato (27), may also be tolerant to nematode attack (19). Presumably, roots of such plants would not suffer ethylene-induced growth inhibition brought on by *H. avenae* infection, but they might be impaired by elevated ABA levels.

The cause of the increase in ABA and ethylene in nematode-infected root tips is uncertain. Probably ABA was not of nematode origin, since ABA assays carried out on homogenized fractions of *H. avenae* J2 were negative (data not shown). Since ethylene production has been shown to increase in a wide variety of plant tissues in response to wounding (20,26), ABA levels may rise in response to an increase in ethylene production following nematode invasion. Alternatively, the elevated concentration in nematode-infected tissue may be indicative of a response induced by the nematode itself. A closer examination of the time course of production of these two growth regulators following inoculation would be useful in that regard.

While this study demonstrated a relationship between nematode invasion and increased levels of ethylene and ABA, there

was no evidence to suggest that increased levels of these hormones were the reason for intolerance. Further work is needed to determine whether tolerant and intolerant plants differ in the effect of nematode invasion on the action of auxin and other shoot-derived factors, what triggers the increase in ABA and ethylene, and whether their effect on growth is additive or synergistic.

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