Effects of Plant Growth Regulators on Development of Pierce's Disease Symptoms in Grapevine

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ABSTRACT

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Foliar applications of indoleacetic acid (IAA) at 200 μg/ml and kinetin at 1,000 μg/ml to *Vitis rotundifolia* 'Carlos,' moderately resistant to Pierce's disease (PD), prevented the development of PD symptoms in inoculated plants in the greenhouse. These treatments also prevented accumulation of PD bacteria in leaves of the plants. In *V. vinifera* 'Carignane,' a highly susceptible cultivar, growth regulators did not prevent PD symptoms. In contrast, ethephon and abscisic acid slightly enhanced symptom development in Carlos and in the highly resistant *V. rotundifolia* 'Higgins.'

Additional key words: xylem-limited bacteria

In the southeastern United States, Pierce's disease (PD) is a limiting factor in the commercial production of the high-quality bunch grapes (*Vitis vinifera* L. and *V. labrusca* L.) (8,11). Because of PD, the grape industry is based on resistant *Vitis* species native to the region, including muscadine (*V. rotundifolia* Michx.) and bunch grapes developed in Florida (*Vitis* spp.) (6). At present, PD is controlled only by this resistance (5).

The causal agent of PD, a xylem-limited bacterium (XLB), was not detected in current-season grapevine tissue during the first 4 wk after budbreak in a Florida vineyard (4). The bacterium apparently does not multiply and accumulate in actively growing juvenile tissue. Symptoms of the disease develop acropetally (5).

In some grape cultivars, symptoms do not develop until fruit maturation. Stress of fruit production was postulated to contribute to disease development, possibly by affecting hormone balance in the vine (7). In peach trees affected by phony disease, also caused by XLB, treatment with gibberellic acid resulted in partial remission of symptoms (3). Because of these observations, the effects of growth-regulator treatments on PD development were evaluated.

MATERIALS AND METHODS

Plant material. Green grapevine cuttings were collected from healthy mother vines maintained in a greenhouse. Cuttings were rooted in a mist bed and grown in a mixture of soil and peat (2:1) in 16-cm-diameter plastic pots. Rooted cuttings with a minimum of six nodes at inoculation were used in the tests. Grape cultivars used in the test included *V. vinifera* 'Carignane,' highly susceptible to PD; *V. rotundifolia* 'Carlos,' moderately resistant; and *V. rotundifolia* 'Higgins,' resistant.

PD strains. Virulent PD strains originally isolated from *V. rotundifolia* were used. Single-colony strains were cloned twice and stored on silica gel to maintain virulence (10). A single PD strain was used to obtain the data presented in this report, but two other strains gave similar results when tests were repeated. For inoculum preparation, the strains were grown at 28 C on a modified PD2 medium (2).

Growth-regulator treatments. Indoleacetic acid (IAA), indolebutyric acid (IBA), benzylaminopurine (BAP), gibberellic acid (GA), and abscisic acid (ABA) were each dissolved in 2–5 ml of dimethyl sulfoxide (DMSO) and diluted with water to the desired concentration. Kinetin was dissolved in 2 ml of dilute hydrochloric acid solution and diluted with water. Ethephon was applied as a dilution of a 21.6% solution. Growth regulators were applied as foliar sprays to thoroughly wet the leaves. Applications were made on a 14-day schedule beginning 7 days before inoculation with the PD bacterium. Each experiment was performed at least twice with three replicates per treatment. Also, each treatment was applied to two uninoculated control plants to assess phytotoxicity.

Inoculation and disease rating. For inoculations, bacteria from cultures 4–6 days old grown on modified PD2 medium (2) at 28 C were suspended in SCP buffer, composed of disodium succinate (1 g/L), trisodium citrate (1 g/L), K_2HPO_4 (1.5 g/L), and KH_2PO_4 (1 g/L), pH 7.0. The inocula were adjusted to an optical density of 0.25 at 600 nm (10^7–10^8 colony-forming units per milliliter) in a Spectronic 2000 spectrophotometer.

Rooted cuttings of the grapevine cultivars were inoculated using a pinprick inoculation technique. Single drops (0.02 ml) of inoculum were placed on the first and third internodes from the base of the plant, and the stem was pierced three to five times through each drop with a dissecting needle. Drops were pulled into the plant by the negative pressure of the pierced xylem vessels. Inoculated plants were maintained in a greenhouse at 28–33 C in the daytime and 20–25 C at night.

Inoculated plants were rated for visible symptoms of PD every 2 wk for 6 mo after inoculation. PD symptoms were confirmed by isolating the PD bacterium in culture from petioles (1). After 6 mo, disease ratings were made on a scale of 0–5, where 0 = no symptoms; 1 = any symptom of PD, such as marginal necrosis (MN) in basal leaf; 2 = MN in one third or less of leaves; 3 = MN in one-third to one-half of leaves; 4 = one-half to three-fourths of leaves with MN and a dead growing point; and 5 = 100% of leaves with symptoms or dead plant.

Determination of bacterial concentration in petioles. Petiole segments 2 cm long were surface-sterilized in 1% sodium hypochlorite for 3 min and washed in four changes of sterile water. They were then ground in 10 ml of SCP buffer with a mortar and pestle, filtered through cheesecloth, and centrifuged to concentrate the bacteria. The bacteria were resuspended in 2 ml of SCP buffer and quantified by dilution plating on modified PD2 medium. Values given are the average of three petioles.

In vitro effect of growth regulators on PD bacterial growth. Concentrated growth-regulator solutions were prepared as described before and filter-sterilized. Growth regulators were added to the modified PD2 medium before pouring. Final concentrations tested per milliliter of media were IAA at 20 and 200 μg, kinetin at 100 and 1,000 μg, ethephon at 40 and 400 μg, GA at 100 and 1,000 μg, and ABA at 10 and 100 μg. Bacterial growth was evaluated by measuring diameters of single, isolated colonies 7 days after suspensions were spread over the medium.

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RESULTS

In Carlos grapevines, IAA at 200 μg/ml prevented PD symptom development in a 6-mo greenhouse test (Table 1). Kinetin also prevented symptom development in four of five plants, and GA, in two of five. PD bacteria could not be isolated from the petioles of symptomless plants that had been treated with IAA or kinetin. In the kinetin- or GA-treated grapevines that did become diseased, symptoms were as severe and bacterial concentrations as high as in the untreated Carlos vines. In this test, ethephon and ABA had no effect on symptoms or bacterial concentration. However, in a subsequent test with Carlos, PD symptoms developed earlier and were more severe in plants sprayed with ethephon or ABA than in control plants. Otherwise, repetition of experiments gave similar results and data presented are representative.

In highly susceptible *V. vinifera* 'Carignane,' PD was not prevented by IAA or kinetin treatments, even at rates higher than those effective on Carlos (Table 2). However, disease severity appeared to be reduced by IAA treatments. In Carignane, IAA was phytotoxic at 200 and 1,000 μg/ml, causing marginal necrosis of leaves and plant dieback similar to PD symptoms. This made disease rating difficult.

Since ethephon and ABA appeared to enhance PD symptoms in Carlos in one test, they were applied to Higgins, which rarely has PD symptoms. Symptoms of PD developed in Higgins sprayed with ethephon at 1,000 μg/ml or ABA at 400 μg/ml (Table 3). Symptoms in treated Higgins were mild when compared with those in Carignane or Carlos. PD bacteria could be isolated from plants with symptoms but not from symptomless ones. Concentrations of PD bacteria in Higgins were not determined.

None of the growth regulators prevented growth of the PD bacterium on modified PD2 medium. Growth was slightly slower on media with IAA at 200 μg/ml or ethephon at 400 μg/ml than on the control medium with no added growth regulator.

DISCUSSION

Clearly, growth regulators can reduce or prevent symptom induction by the PD bacterium in Carlos grapevines grown in the greenhouse. Carlos vines growing in a nearby vineyard develop marginal necrosis of leaves and some dieback of growing points each year, but they have sufficient tolerance to survive and continue to produce fruit for several years. Carignane, in which growth regulators did not prevent PD symptoms, is highly susceptible to PD and does not survive in the same vineyard. Growth regulators may be useful in controlling PD in grape cultivars that already have moderate tolerance to PD, or perhaps the growth-regulator effect is limited to *V. rotundifolia* and does not occur in *V. vinifera*. Further tests with large vines in the vineyard are required to determine the usefulness of growth regulators for control of PD in commercial vineyards.

Unlike the use of GA on phony disease of peach, where disease symptoms were reversed without affecting XLB populations (3), IAA and kinetin not only prevented symptoms but prevented the accumulation of PD bacteria in leaves of Carlos. Since none of the growth regulators inhibited growth of the PD bacterium in vitro, the effect must have

Table 1. Effect of plant growth regulators on development of Pierce's disease (PD) symptoms in *Vitis rotundifolia* 'Carlos'

Growth regulator[a]	Conc. (μg/ml)	Diseased/ inoculated[b]	Disease rating (0–5)[c]	Bacterial conc.[d] (cells/cm of petiole)
Indoleacetic acid	200	0/5	0	0
Kinetin	1,000	1/5	4	0
Gibberellic acid	1,000	3/5	4	ND
Ethephon	1,000	5/5	4	1.4×10^6
Abscisic acid	100	4/5	3	2.1×10^6
None	···	5/5	4	1.0×10^6

[a] Growth regulators were applied as foliar sprays on a 14-day schedule beginning 7 days before inoculation.
[b] Number of Carlos plants with PD symptoms over total number of inoculated plants.
[c] Six months after inoculation, disease severity was rated on a scale of 0–5: 0 = no symptoms, 1 = marginal necrosis (MN) in basal leaf, 2 = MN in one-third or less of leaves, 3 = MN in one-third to one-half of leaves, 4 = MN in one-half to three-fourths of leaves and a dead growing point, and 5 = 100% of leaves with symptoms, or dead plant.
[d] Bacterial concentrations per centimeter of petiole were determined by dilution plating 6 mo after inoculation. Leaves with MN were used, except for the indoleacetic acid and kinetin treatments, where symptomless lower leaves were used. ND = not determined.

Table 2. Effect of plant growth regulators on development of Pierce's disease (PD) symptoms in *Vitis vinifera* 'Carignane'

Growth regulator[a]	Conc. (μg/ml)	Diseased/ inoculated[b]	Disease rating (0–5)[c]	Bacterial conc.[d] (cells/cm of petiole)
Indoleacetic acid	200	6/6	4	1.7×10^7
	1,000	3/3	3	2.9×10^7
Kinetin	1,000	6/6	5	ND
	2,000	3/3	5	ND
Indolebutyric acid	200	3/3	5	ND
Benzylaminopurine	1,000	3/3	5	7.0×10^6
None	···	6/6	5	2.4×10^7

[a] Growth regulators were applied as foliar sprays on a 14-day schedule beginning 7 days before inoculation.
[b] Number of Carignane plants with PD symptoms over total number of inoculated plants.
[c] Six months after inoculation, disease severity was rated on a scale of 0–5: 0 = no symptoms, 1 = marginal necrosis (MN) in basal leaf, 2 = MN in one-third or less of leaves, 3 = MN in one-third to one-half of leaves, 4 = MN in one-half to three-fourths of leaves and a dead growing point, and 5 = 100% of leaves with symptoms, or dead plant.
[d] Bacterial concentrations per centimeter of petiole were determined by dilution plating 6 mo after inoculaton. Leaves with MN were used. ND = not determined.

Table 3. Effect of ethephon and abscisic acid on development of Pierce's disease (PD) symptoms in *Vitis rotundifolia* 'Higgins'

Growth regulator[a]	Conc. (μg/ml)	Diseased/ inoculated[b]	Disease rating (0–5)[c]
Ethephon	100	0/3	0
	250	0/3	0
	1,000	2/3	2
Abscisic acid	100	0/3	0
	400	2/3	1
None	···	0/3	0

[a] Growth regulators were applied as foliar sprays on a 14-day schedule beginning 7 days before inoculation.
[b] Number of Higgins plants with PD symptoms over total number of inoculated plants.
[c] Six months after inoculation, disease severity was rated on a scale of 0–5: 0 = no symptoms, 1 = marginal necrosis (MN) in basal leaf, 2 = MN in one-third or less of leaves, 3 = MN in one-third to one-half of leaves, 4 = MN in one-half to three-fourths of leaves and a dead growing point, and 5 = 100% of leaves with leaf symptoms, or dead plant.

been on the host. The mechanism through which the growth regulators inhibit the PD bacterium and symptom development in Carlos is not known. IAA, kinetin, and GA provided various levels of disease suppression and also retarded foliar senescence (9). In contrast, ethephon (an ethylene-generating chemical) and ABA appeared to increase symptom development slightly in Carlos and Higgins. These growth regulators are known to accelerate senescence. PD symptoms do not develop and the PD bacterium does not occur in young, juvenile grapevine tissue. Perhaps, IAA and kinetin prevented PD development by retarding foliar senescence, thus maintaining Carlos vines in a more tolerant growth stage. Studies are under way to elucidate the mechanism of PD control by IAA and kinetin.

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