Prevention of Black Spot Disease in Persimmon Fruit by Gibberellic Acid and Iprodione Treatments

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ABSTRACT

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Black spot disease (BSD) in persimmon fruit, caused by *Alternaria alternata,* develops primarily in the high humidity environment beneath the calyx. Three treatments with gibberellic acid (GA_3, 20 μg/ml) applied during fruit development at 30, 20, and 10 days before harvest, reduced decay more effectively than the single commercial treatment of GA_3 (50 μg/ml) applied 10 days before harvest. As a result of GA_3 treatment, the calyx of the fruit remained erect till harvest. The correlation coefficent between BSD under the calyx and calyx erectness was $r = -0.72$. Persimmon fruits treated with GA_3 in the orchard and stored at 0 C for 3 mo showed less fruit area becoming covered with black spot during storage compared with untreated fruit. Increasing the number of sprays from one to three resulted in a decreased infected area. Sprays of GA_3 also inhibited fruit softening, with a correlation coefficient of $r = -0.63$ between decayed area and fruit firmness. GA_3 at concentrations up to 200 μg/ml had no effect on fungal development in vitro and in vivo on inoculated fruits. GA_3 apparently affects decay development by enhancing resistance of the fruit. Preharvest treatment with GA_3 and the fungicide iprodione further reduced the percentage of fruits unmarketable due to BSD.

Alternaria alternata (Fr.) Keissl. causes the black spot disease (BSD) in persimmon (*Dyospyros kaki* L.) (10). Under high humidity, the pathogen penetrates the fruit directly or through wounds, during fruit growth. Direct penetration can occur during the entire growth period of the fruit, but BSD usually develops after harvest. Black spot symptoms generally occur after 10 wk of storage at −1 C. Wound penetration occurs mostly through small cracks, which occur on the fruit shoulder beneath the calyx. These wounds appear as the fruit approaches harvest maturity and are the site of preharvest disease symptoms, which are dependent on heavy rainfall. Decay incidence at harvest is virtually absent when the season is dry, as frequently occurs with diseases caused by *Alternaria* spp. (11). When disease develops during the harvest season, black spot symptoms appear almost entirely beneath the calyx and they are more prominent on riper fruits in the orchard. Disease development on stored fruit involves the entire fruit surface.

Treatments with protective fungicides such as maneb, applied in the orchard, have not been successful in reducing decay development beneath the calyx (D. Prusky and R. Ben-Arie, *unpublished data*). Disease was reduced on the exposed fruit surface, but only about 5% of this area ever develops BSD. The main infected area is beneath the calyx, where 65–75% of the area is often diseased at harvest. Decay symptoms may reduce the marketability of the crop by up to 50%, depending on the prevalent weather conditions.

To improve the storage quality of persimmon fruits, a preharvest GA_3 treatment of 50 μg/ml is often applied as an orchard spray 10–14 days before harvest (3,4). This treatment slightly reduces fruit size (3,4) but extends the storage life of the fruit by delaying fruit softening. We have observed that the calyx lobes of many of the GA_3-treated fruits remain erect until harvest. Taking into account the importance of high humidity beneath the calyx for fungal infection, the aim of this work was to study the effect of various GA_3 applications, occasionally with the addition of the fungicide iprodione, on disease incidence in order to improve the control of preharvest and postharvest BSD.

MATERIALS AND METHODS

Field experiments. Trials were carried out during 1990–1991 in six persimmon (*Dyospyros kaki* L. cv. Triumph) orchards, in the coastal plain of Israel. Ten-year-old uniform trees from an orchard of Kibutz Hatzor with about 500 fruits per tree were used as treatment units. The effect of gibberellic acid (GA_3) alone on persimmon fruits was tested in one orchard of Kibutz Hatzor, using a randomized block design with five replications. One to three sprays of GA_3 (Pro-Gibb 4%, Abbott Laboratories, USA) were applied at 0, 10, 20, and 30 μg/ml. Treatments were applied 30, 20, and 10 days before harvest. Single treatments were applied 30 days, two treatments were applied 30 and 20 days before harvest. Three treatments were applied 30, 20, and 10 days before harvest. Harvesting was carried out according to commercial practice, when fruit color had changed from green to light orange. The experiment was repeated twice during 1990 and 1991 and the data analyzed separately for each year. The factorial part of three GA doses by three frequencies was analyzed by single-degree-of-freedom partitions of linear and quadratic effects and their interactions. A further single-degree-of-freedom contrast between the control and the average of treated fruits was also tested.

The combined effect of GA_3 and fungicide on decay development was tested during the 2 consecutive years in a total of six sites by using a split-plot experimental design with five replications in each case. All the orchards were selected in the coastal plain since it is the area most affected by the disease. The orchards were distributed in two main groups: in the south of the coastal plain, Ganei Hadar and Havat Tzrifim, and in the center-north of the coastal plain, Moshav Adanim and Kibutz Ma'barot. In some orchards, the experiment was repeated both years. Trees in the orchard were planted in a row 4 m apart, with 5 m separation between rows. Each experimental unit was composed of three trees in a row and fruit was sampled only from the middle one. The fungicide tested, iprodione (Rovral 50 WP, Rhone-Poulenc, France) (6,8,9), was sprayed two or three times at 1,000 μg/ml a.i., between 40 and 15 days before harvest, superimposed on GA_3-treated trees. GA_3, at 20 μg/ml, was sprayed 30, 20, and 10 days before harvest. The commercial GA_3 treatment of a single 50 μg/ml spray was applied 10 days prior to harvest to compare with the effect of the split GA_3 treatment on preharvest calyx erectness and postharvest fruit softening. Fungicide treatments were applied to the trees treated three times with GA_3 and those treated commercially. The fungicide treatments were always applied one day after the GA_3 treatment. Control trees received no sprays. Fungicide and GA_3 were applied by means of a grove sprayer equipped with two hand-operated spray guns at a pressure of 20 kg/cm^2. Each tree was sprayed with 7–8 L of spray mix. During 1990, four field experiments were carried out; during 1991, only two. Not all treatment combinations were applied in all experiments. The factorial part of the experiments, including the two levels of GA_3 (one spray of 50 μg/ml and three sprays of 20 μg/ml), two levels of iprodione treatment (treated and untreated), and control (untreated fruits), is common to all experiments and will be presented here. The factorial part of the experiments was analyzed separately for each experiment and year. In general, similar trends were found for the GA and iprodione effects in different experiments. Therefore all the data from experiments of each particular year, either at harvest or during storage, were analyzed together with weights proportional to the inverse of variance estimates found in the separate analyses.

Assessment of fruits at harvest. The area covered by BSD was estimated by comparison with a diagramatic scale depicting different areas covered by the disease. The main area covered by the disease lies beneath the calyx, which covers about 18% of the fruit surface area. Fruit with a decayed area exceeding 1% was considered unmarketable. Calyx erectness (CE) was evaluated on a 0–4 scale. Zero was assigned to a calyx adjacent to the fruit surface and index 4 was used to describe a calyx with all sepals at an angle of at least 75° (Fig. 1). Fifty fruits were evaluated at harvest from each tree (treatment unit) and a total of 250 fruits per treatment (50 fruit × 5 replications) was assessed.

Assessment of fruit after storage. For storage experiments, 30 fruits per replicate, with less than 1% decayed area were stored at −1 C. After 5 mo, the area covered by BSD was evaluated as above. Fruit firmness was assessed by response to pressure, based on an index of 0 (soft) to 2 (hard), as described by Guelfat-Reich and Ben-Arie (7).

Effect of GA_3 on *Alternaria alternata* development. Five-millimeter diameter hyphal disks of *Alternaria alternata* obtained from a freshly growing colony were placed on potato-dextrose agar (PDA) amended with GA_3. Inoculated plates were incubated at 20 C and development was observed daily. Each treatment was repeated 15 times.

Fruits sprayed with 20 μg/ml GA_3 three times during their development were used to test the development of *Alternaria alternata* following artificial inoculation. Inoculation was done by forming a 2-mm-deep × 1-mm-wide wound with a pin on four sides of the fruit. Five microliters of the spore suspension of 10^3 and 10^4 conidia per milliliter were placed on the wounds. Fruits were incubated at 17 C and 95% RH. Data were analyzed by a generalized linear model comparing the linear rate of infection development of the different levels of spore suspension treatments.

RESULTS

Effect of frequency and concentration of GA_3 treatment on BSD and CE at harvest. The area of BSD decreased signficantly ($P \leq 0.01$) by GA_3 applications compared with the control. As the number of GA_3 sprays increased from one to three (Fig. 2A), the area of BSD decreased linearly ($P = 0.067$). The differences between the GA_3 doses of 10, 20, and 30 μg/ml were not significant.

CE of the GA_3-treated fruits increased compared with the control ($P \leq 0.01$). As the number of GA_3 sprays increased from one to three, only the 20 μg/ml GA_3 dose showed an increase

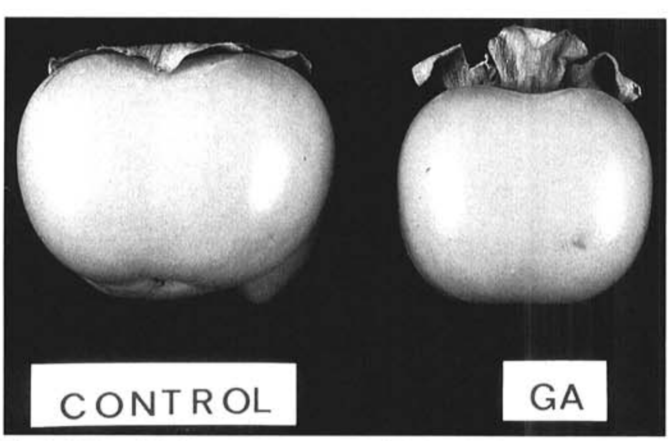

Fig. 1. Effect of GA_3 on calyx erectness of fruits of Triumph persimmon *a.* untreated fruits, *b.* three treatments with 20 μg/ml GA_3.

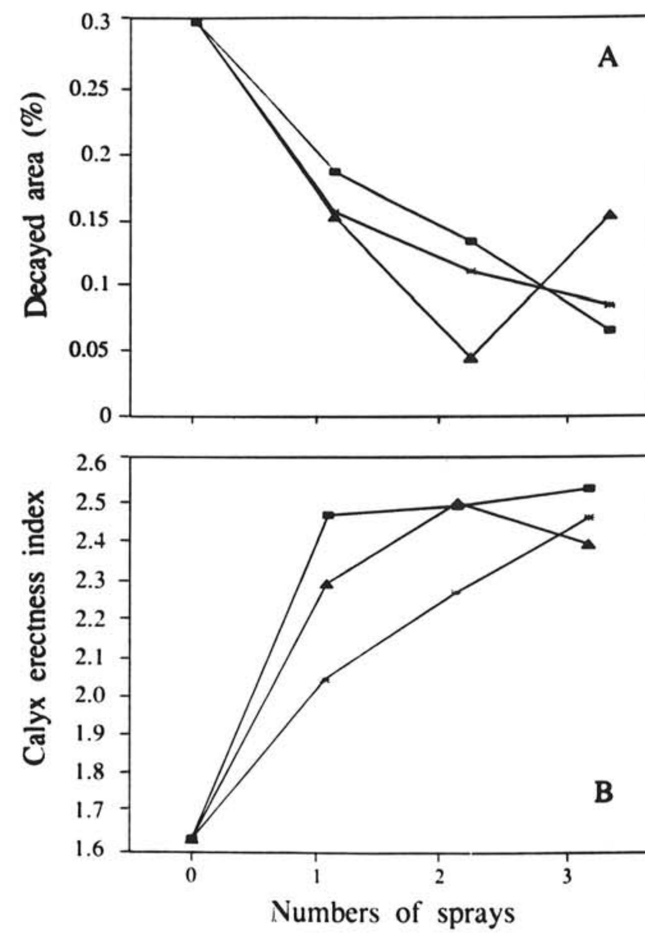

Fig. 2. Effect of GA_3 treatments during fruit development on the percentage of fruit surface area with lesions caused by *Alternaria alternata* (**A**) and calyx erectness (**B**) on Triumph persimmon fruits at harvest. The three spray treatment was applied 30, 20, and 10 days before harvest; the two spray treatment 30 and 20 days before harvest; the single spray treatment 30 days before harvest. Sprays were at three concentrations: 10 μg/ml (■), 20 μg/ml (*) and 30 μg/ml (▲). Decayed area was estimated by comparison with diagrams of percent coverage. Calyx erectness varies between 0 at 4 depending on the angle between sepals and fruit according to Materials and Methods. Each point is the average of values of 250 fruits (50 fruits × 5 replications).

in CE (Fig. 2B), but overall no significant effect on CE was observed from the increase in number of GA_3 sprays from one to three. The correlation between CE index and the percentage of decayed area, calculated from the first year of experiments, was $r = -0.724$ (Fig. 3). Similar results were obtained during the second year of experiments (data not presented).

Effect of frequency and concentration of GA_3 treatment on BSD and fruit firmness during storage. Decay development on fruit treated with 10, 20, and 30 μg/ml GA_3 after 7 mo of storage decreased as the number of sprays increased from zero to three. The linear effect of the number of GA_3 sprays on the decayed area was highly significant ($P = 0.008$). However, no significant effect of the different GA_3 concentrations was observed (Fig. 4A).

Firmness of the fruit was also affected by all GA_3 treatments compared with untreated fruits ($P \leq 0.01$) (Fig. 4B). The linear effect of the number of sprays of GA_3 (from one to three) on the firmness of the fruit was highly significant ($P \leq 0.0001$). Also, the interaction between the linear effect of the number of sprays of GA_3 and the quadratic effect of GA_3 dose was significant ($P = 0.018$) because of the different curvature of the lowest GA_3 dose (10 μg/ml GA_3) from the other dosages. When firmness values were correlated with decay development a correlation coefficient of $r = -0.63$ was obtained.

Effect of GA_3 on fungal growth and infectivity. No differences were observed in the rate of fungal development at different concentrations of GA_3 from 5 to 200 μg/ml when amended into PDA. There was also no difference in the extent of fungal development on inoculated fruits treated with GA_3 compared with that on control fruits (Fig. 5). Diameter of decay development, however, increased linearly ($P \leq 0.01$) with the increase of spore concentration. The slope of the regressions of diameter of decay on the number of days after inoculation when fruits were inoculated with 10^4 and 10^3 spores per milliliter was 3.56 and 2.4, respectively; when fruits were uninoculated, it was 0.2. All the slopes at the different spore concentrations differed significantly ($P \leq 0.01$). No significant differences were observed between the slopes of GA_3 and untreated fruits at each concentration of spores.

Effect of GA_3 and iprodione treatments on quality parameters at harvest and during storage. Any of the GA_3 treatments alone or combined with iprodione significantly increased the CE index, reduced the decayed area and the number of unmarketable fruits, and increased fruit firmness when compared with untreated fruits (Tables 1 and 2). In 1990 experiments, the CE index was significantly higher with three sprays of 20 μg/ml GA_3 than with the single commercial treatment of 50 μg/ml GA_3. In 1991 experiments, this effect was not observed.

Similarly, in 1991 experiments, firmness index values were significantly greater when fruit was treated with three GA_3 treatments

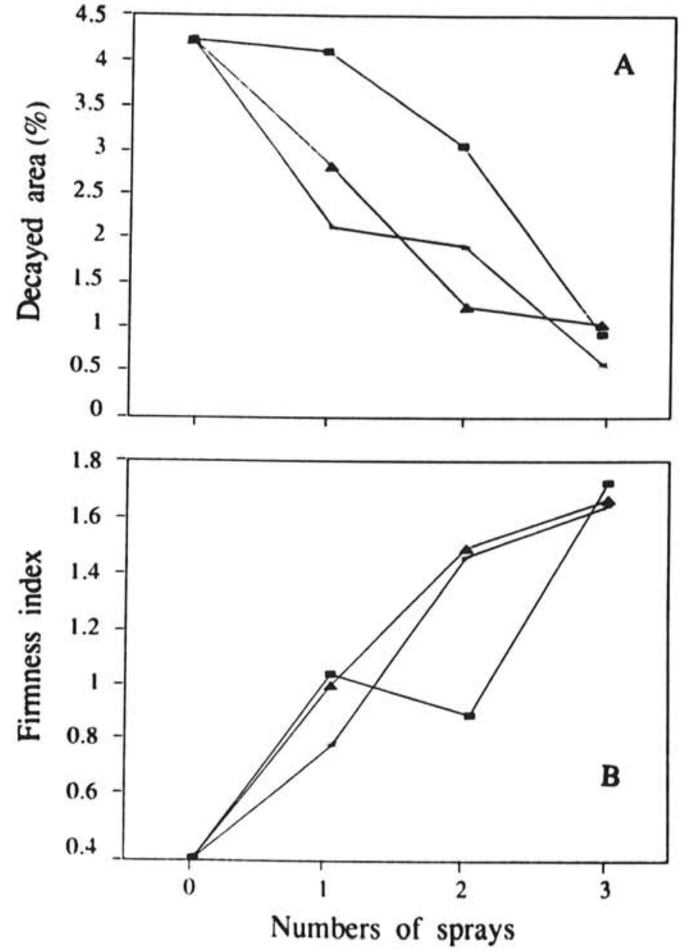

Fig. 4. Effect of GA_3 treatments before harvest on percentage of fruit surface area with lesion caused by *Alternaria alternata* (**A**) and firmness of Triumph persimmon fruits (**B**) after storage for 7 mo at −1 C. Three GA_3 sprays were applied 30, 20, and 10 days before harvest. Two-spray treatment was applied 30 and 20 days before harvest. The single-spray treatment was applied 30 days before harvest. GA_3 was applied at 10 μg/ml (■), 20 μg/ml (*) and 30 μg/ml (▲). Each point represents the average 250 fruits (50 fruit × 5 replications). Percentage of decayed area was estimated by comparison of the decayed area with diagrams of percent coverage. Index of firmness varies between 0 and 2 as described in Materials and Methods.

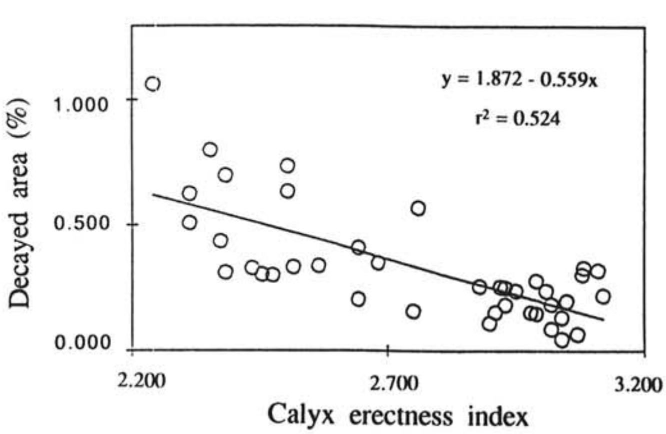

Fig. 3. Relationship between percentage of decayed area by *Alternaria alternata* and calyx erectness index on Triumph persimmon fruits at harvest in the orchard of Kibutz Hatzor. Each point is the average of values of 50 fruits per treatment that had been treated 0, 1, 2, or 3 times with GA_3 at either 10, 20, or 30 mg/ml. during 1990 as described in Fig. 2.

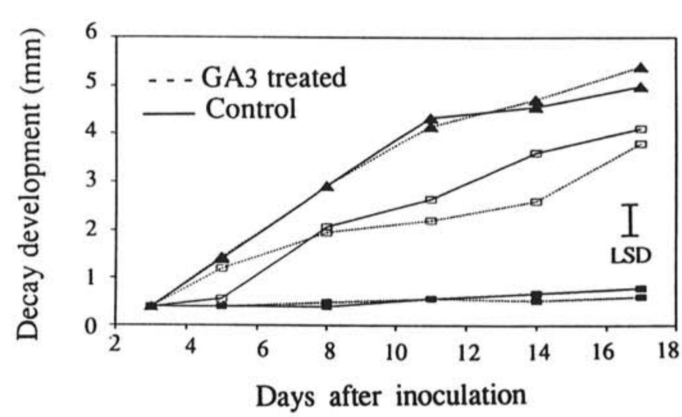

Fig. 5. Decay development of wounded Triumph persimmon fruit treated with GA_3 and inoculated with *Altenaria alternata* spores. Each treatment represents 15 fruits that were wounded and inoculated with 5 μl of water (■) or a suspension of 10^3 (▲) and 10^4 (□) spores per milliliter in four sites of the distal portion of the fruit. Vertical band denotes LSD ($P \leq 0.05$).

compared with the single commercial GA_3 spray. In 1992 experiments, however, this effect was not observed.

A consistent reduction of decayed areas as an effect of iprodione was observed in all experiments, although it was significant only in 1992 at harvest time. To a somewhat smaller degree, the treatment of three sprays with GA_3 reduced, in most cases, the decayed area more than the single commercial GA_3 treatment. However, this effect was never significant. The percentage of unmarketable fruits was reduced when iprodione was applied in all experiments. This effect was significant in three of the four analyses (Tables 1 and 2).

Differences between experiments were in most cases significant, but no significant interactions were found (except for one) between experiments and treatments. This fact justifies the pooling of experiments in any one year to a joint weighted analysis.

DISCUSSION

BSD caused by *A. alternata* is the main postharvest disease of persimmon in Israel (10). Treatments with protective fungicides used in the past did not control the disease at harvest. Various causes were suggested for this failure: *1)* the lack of penetration of the fungicide beneath the calyx, where most of the black spot occurs; *2)* abundant rains, which sometimes occur during the harvest season, generate a micro-environment beneath the calyx producing optimal conditions for fungal development (11) accompanied by removal of any applied fungicide; and *3)* increased susceptibility of the region beneath the calyx as a result of crack formation during fruit growth and the expansion of these wounds under continuous rains. Our approach to the control of the disease was aimed at enhancing the natural resistance of the fruit to

TABLE 1. Percentage of fruit surface with lesions caused by *Alternaria alternata*, unmarketable fruits, and calyx erectness index at harvest in Triumph persimmon after field treatment with GA_3 and iprodione at harvest

	1990			1991		
Treatment	Decayed area (%)	Unmarketable fruits (%)	CE index[a]	Decayed area (%)	Unmarketable fruits (%)	CE index
Control	0.53[b]	9.0[b]	1.60[b]	0.74[b]	17.9[b]	2.41[b]
1 × 50 μg GA_3/ml	0.36	5.5	1.78	0.79	18.1	2.74
3 × 20 μg GA_3/ml	0.34	5.9	2.00	0.47	9.1	2.69
1 × 50 μg GA_3/ml + iprodione[c]	0.32	5.0	1.72	0.41	7.1	2.68
3 × 20 μg GA_3/ml + iprodione[c]	0.31	5.1	1.90	0.30	4.9	2.67
Source			Levels of significance[d]			
GA_3	ns[e]	ns	*	ns	ns	ns
Ip	ns	ns	ns	*	*	ns
GA_3 × Ip	ns	ns	ns	ns	ns	ns
Exp	**	**	*	ns	ns	ns
GA_3 × Exp	ns	ns	ns	ns	ns	*
Ip × Exp	ns	ns	ns	ns	ns	ns
GA_3 × Ip × Exp	ns	ns	ns	ns	ns	ns
Untreated vs. treated	**	*	*	**	**	*

[a]Calyx erectness (CE) varies between 0 and 4 depending on the angle between sepals and fruit surface as described in Materials and Methods.
[b]Mean values for different treatments.
[c]Two sprays at a concentration of 1,000 μg/ml were applied between 40 and 15 days before harvest.
[d]The levels of significance are of the different effects and interactions. Four orchards were treated in 1990: Ganei Hadar, Havat Tzrifim, Moshav Adanim, and Kibutz Ma'barot. Two orchards were treated during 1991: Havat Tzrifim and Kibutz Ma'barot.
[e]$P = 0.05$ (*); $P = 0.01$ (**); nonsignificant (ns).

TABLE 2. Percentage of fruit surface with lesions caused by *Alternaria alternata*, unmarketable fruits, and firmness in Triumph persimmon after treatment with GA_3 and iprodione and storage for 5 mo at −1 C after treatment with GA_3 and iprodione

	1991			1992		
Treatment	Decayed area (%)	Unmarketable fruits (%)	Firmness index[a]	Decayed area (%)	Unmarketable fruits (%)	Firmness index
Control	2.60[b]	46.1[b]	1.40[b]	3.62[b]	82.2[b]	0.13[b]
1 × 50 μg GA_3/ml	1.54	28.4	1.54	2.69	68.3	0.40
3 × 20 μg GA_3/ml	1.21	23.0	1.69	1.60	46.7	0.50
1 × 50 μg GA_3/ml + iprodione[c]	0.90	13.3	1.49	1.55	39.4	0.44
3 × 20 μg GA_3/ml + iprodione[c]	0.76	17.5	1.63	1.85	44.8	0.26
Source			Levels of significance[d]			
GA_3	ns[e]	ns	*	ns	ns	ns
Ip	ns	*	ns	ns	*	ns
GA_3 × Ip	ns	ns	ns	ns	*	ns
Exp	*	**	**	*	**	ns
GA_3 × Exp	ns	ns	ns	ns	ns	ns
Ip × Exp	ns	ns	ns	ns	ns	ns
GA_3 × Ip × Exp	ns	ns	ns	ns	ns	ns
Untreated vs. treated	**	**	**	**	**	**

[a]Fruit firmness was assessed based on an index of 0 (soft) to 2 (hard).
[b]Values at each year are the mean values for all the fruit from treated orchards during the winter season of 1990 and 1991 followed by storage until spring 1991 and 1992 respectively.
[c]Two sprays at a concentration of 1,000 μg/ml were applied between 40 and 15 days before harvest.
[d]The levels of significance are of the different effects and interactions. Four orchards were treated in 1990: Ganei Hadar, Havat Tzrifim, Moshav Adanim, and Kibutz Ma'barot. Two orchards were treated during 1991: Havat Tzrifim and Kibutz Ma'barot.
[e]$P = 0.05$ (*); $P = 0.01$ (**); non significant (ns).

disease development and increasing fungicide efficiency.

Alternaria infection is strongly dependent on high humidity. The high incidence of the disease beneath the calyx may be the result of the persistent high humidity, compared with other exposed parts of the fruit. This can be easily observed in the orchard in the early morning. It was observed that in general GA_3 treatment enhanced CE and consequently no large area of BSD developed. This is evident by the fairly high correlation ($r = -0.72$) between CE and disease reduction. The disease incidence at harvest was not significantly affected by the number of sprays or GA_3 concentration. This might indicate that the effect on CE and the possible consequent decrease in local humidity on the fruits were insufficient to control disease development. GA_3 reduces significantly the BSD infected area during storage on fruits that were initially selected as free of disease symptoms at harvest; this fact supports the possible direct involvement of GA_3 in the prevention of disease development. Ben Arie and coworkers (3–5) reported that GA_3 delays fruit softening (3–5). However, when this factor was correlated with the incidence of decay, a correlation value of $r = -0.63$ was obtained, suggesting that fruit firmness is not the main cause for inhibition of decay development. These experiments indicate that GA_3 has no direct effect on the pathogen. The effect of GA3 on fungal development could possibly be a physiological one, delaying fruit senescence or healing wounded tissue.

It is possible that the cracks that occur beneath the calyx during fruit development are a symptom of fruit senescence and their appearance is retarded by GA_3, as was suggested by Aharoni et al in the case of lettuce (1) and Barkai-Golan and Aharoni in celery (2). In the first case, a single preharvest spray of 25 μg/ml of GA_3 caused a significant decrease in decay caused by *Stemphylium* spp. and *Bremia lactucae,* attributed by the authors to the marked effect of retardation of senescence of lettuce leaves and preservation of the juvenility of the tissues. Iprodione as a preharvest treatment was capable of significantly reducing the percentage of unmarketable fruits by BSD before and after harvest. This effect was more clearly observed after harvest, but in 1991 it was also observed before harvest. Iprodione had an effect on the number of unmarketable fruits but a significant reduction of the percentage of unmarketable fruits was observed in only one year, because GA_3 already reduced disease incidence.

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