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FULL RESEARCH PAPER

Improved control of moldy-core decay (*Alternaria alternata*) in Red Delicious apple fruit by mixtures of DMI fungicides and captan

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Abstract The sterol biosynthesis inhibitors bromuconazole and difenoconazole and tank mixes of each fungicide with captan were applied to apples and evaluated as controls for moldy-core and fruit decay caused by *Alternaria alternata*. Effectiveness of a mixture of bromuconazole and captan in controlling colonization by the fungus was also evaluated. Decay formation by *A. alternata* on mature detached fruits was partially inhibited by bromuconazole at 0.5 μg ml^{-1} and was completely inhibited at 50 μg ml^{-1}; it was significantly affected by either bromoconazole at 5 μg ml^{-1} or captan at 1,250 μg ml^{-1}, and was completely inhibited by their mixture. In general, three foliar applications of bromuconazole or difenoconazole in the field, during the bloom period, reduced the numbers of infected fruits by 40–60% compared with untreated control trees. However, tank mixes of either fungicide with captan improved control of moldy-core in fruits at harvest. Tank mixtures of bromuconazole and captan also significantly reduced the percentage of fruits colonized by *A. alternata* when sampled at various days after full bloom. Artificial inoculations in the orchard at full bloom did not change the inhibitory effects of the tank mixtures. Large-scale demonstration trials in commercial orchards supported these findings. The inhibitory effects of tank mixes on decay development in detached fruits, and on moldy-core in the field indicate that a control programme based on mixtures of either bromuconazole or difenoconazole with captan during the bloom period can effectively reduce moldy-core on Red Delicious apples.

Keywords Disease control · *Malus sylvestris* var. *domestica* · Moldy-core disease · Sterol inhibitors

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Introduction

Alternaria alternata, is the fungal pathogen predominantly responsible for moldy-core in the Red Delicious apple cultivar (Brown and Hendrix 1978; Ellis and Barrat 1983; Reuveni et al. 2002; Teixido et al. 1999). Spores infect the flowers during and shortly after the bloom period (Miller 1959; Reuveni et al. 2002), and mycelia reach the seed and carpel wall during fruit growth and storage (Miller 1959). Moldy-core is characterized by the growth of the mycelia within the loculus (the ovary cavity or seed cavity), with or without penetration into the mesoderm. The disease may become invasive and lead to a slow, dry rot confined to the flesh immediately surrounding the core (Ellis and Barrat 1983; Spotts 1990). External

symptoms are rare, although infected fruits may colour and drop prematurely (Ellis and Barrat 1983; Spotts 1990). Conditions of high relative humidity, mild temperatures during spring, and tissue susceptibility are important factors that affect the natural infection in orchards. Once inside the fruit, the fungus is protected against contact fungicides, and conditions for its growth are excellent.

In recent years levels of moldy-core in Red Delicious have caused significant losses in Israel. The incidence of fruits infected with moldy-core, according to samplings from a packaging house in northern Israel, averaged from 7–12% until 2002, but in 2003 there was a significant increase in disease incidence: up to 40% in some orchards (Reuveni 2006). In general, when more than 8% of the fruits are infected with moldy-core, they can only be sold for industrial use at low prices. The Red Delicious cultivar constitutes about 40% of the apple trees planted in Israel. Therefore, moldy-core is an important factor that reduces apple fruit quality, and can be an economically important problem.

Attempts to control moldy-core by using foliar sprays of several fungicides, e.g. benomyl, captan, dodine, iprodione or mancozeb have been unsuccessful (Ellis and Barrat 1983; Combrink et al. 1985; Biggs et al. 1993). Recently, some other compounds have been reported to act on some species of *Alternaria*, including *A. alternata*, in apple; they included the strobilurins trifloxystrobin (Flint, Bayer CropScience) and azoxystrobin (Abound, Syngenta), and the DMI fungicide difenoconazole (Score, Syngenta) (Reuveni and Sheglov 2002), and potassium phosphite (Reuveni et al. 2003). The activity of two fungicides—the DMI fungicide bromuconazole (Vectra, Aventis) and the recently introduced premix of pyraclostrobin and boscalid (Pristine, BASF) has recently been shown to inhibit *A. alternata* and moldy-core in apple (Reuveni 2006). However, none of these fungicides has yet been found to provide satisfactory control of the disease.

The objective of the present study was to assess the inhibitory effects of bromuconazole and of its tank mixes with the fungicide captan, on the decay of apple fruits. In addition, the efficacy of bromuconazole and difenoconazole and of tank mixes of each with captan, in controlling moldy-core in field-grown Red Delicious apples, following natural infestation or artificial inoculation, was examined.

Materials and methods

Fungicides

The activities and efficacy of the following fungicides were tested: the DMI fungicides, bromuconazole (Vectra, 100 SC, Aventis, France) (Reuveni 2006); and difenoconazole (Score, EC, Syngenta, Basel, Switzerland) (Reuveni et al. 2002), and the protectant fungicide captan (Merpan, 50 WP, Makteshim-Agan, Israel).

Fruits, fungal isolate and inoculation procedure

Alternaria alternata was isolated from fruits of the 'Starking' strain of Red Delicious with moldy-core. The fruits were disinfected with 90% ethanol, and small pieces (1–2 mm) of the mesoderm tissue, external to the core region, that showed moldy-core symptoms, were removed and placed on PDA at 25°C. Single-spore cultures were maintained on PDA plates for 10–12 days until conidia were produced (Reuveni et al. 2002). For inoculation, the assay described previously by Reuveni and Sheglov (2002) was used. In summary, Starking fruits were brought from the orchard, washed with water, blotted dry with paper tissue, sprayed with 90% ethanol, and allowed to dry at room temperature. Each fruit was pricked with a 2 mm diam sterile tip to a depth of 3 mm and inoculated by pipetting 15 µl of conidial suspension containing 5×10^5 spores ml^{-1} dropped onto each wound site. Following inoculation, the fruits were placed on trays containing wet filter paper, covered with plastic bags to maintain high humidity (RH) and held in a growth chamber (25°C, 100–120 μE in m^{-2} s^{-1}, 16 h of light per day) for 7–10 days.

Decay development in detached fruits

To assess the effect of a DMI fungicide, alone or in a mixture with captan, on decay development in detached fruits, an aqueous solution of the DMI fungicide or of its mixture with captan was mixed with an equal amount of conidial suspension, so as to give the desired final concentration of each fungicide (Reuveni 2006). The final inoculum concentration in all the mixtures was 5×10^5 conidia ml^{-1}. A control conidial suspension was mixed with an equal amount of water. Each fruit was pricked to a

depth of 3 mm with a 2 mm diam sterile tip at six sites, and each site was inoculated with 15 µl of an aqueous mixture. Following inoculation, the fruits were incubated in a moist chamber as described above, and held at 25°C for 10 days. The decay lesion diameter produced at each inoculation site was measured every two days. Three fruits were used for each concentration (given as µg a.i. ml^{-1}) of each fungicide or their mixture.

Field trials to evaluate the efficacy of fungicides and mixtures

Field experiments with apples, cvs 'Top Red' and 'Oregon Spur', were conducted in commercial orchards in 2004 and 2005 on the Golan Heights and in the Galilee regions of Israel. These cultivars are susceptible to moldy-core, which had been prevalent in these orchards in previous years. Fertilization, irrigation and other cultural practices were as recommended to commercial growers by the Extension Service of the Israeli Ministry of Agriculture and Rural Development. The annual rainfall in this region is 800–900 mm, all of which occurs during the winter (October–April), and the average midday RH and temperature in summer are 35–40% and 30°C, respectively. The sky is cloudless during most of the summer. Night temperatures occasionally fall to 14–20°C between the end of May and August, and dew may accumulate on the leaf surfaces during some nights. During the spring, temperatures are moderate (10–25°C) and humidity conditions in this region are favourable for fungal infection.

Experimental design

Application of fungicides began at the beginning of bloom (unless otherwise stated). Sprays were applied to run-off (2,500 l ha^{-1}) with a 100 l gun-sprayer (1,400 kPa), at the time intervals specified for each experiment on a 'calendar' and phenological basis. For large-scale demonstration trials a 2,000 l speeder-type sprayer was used to apply 1,500 l ha^{-1}. Spacings between trees were 5 × 3 m or 5 × 4 m, with a buffer row between treatments. Treatments within experiments were arranged in a randomized complete block design. Each plot consisted of four to six trees, with five replications in every experiment.

Efficacy of fungicides—Experiment 1

In 2004, Experiment 1 was undertaken to compare the efficacy of two DMI fungicides and that of a tank mixture of each with captan in controlling moldy-core, and to determine the effects of number and timing of the applications of each of the DMI fungicides alone. This experiment included the following treatments: bromuconazole (0.08% v/v), difenoconazole (0.02% v/v), and a tank mixture of each fungicide with captan (0.25% v/v). The treatments were applied to 15 year-old cv. 'Top Red' trees on MM.104 rootstock, in the Ein Zivan orchard in the Golan region. Three foliar sprays of each fungicide or of their tank mixture were applied, the first on 31st March, 2004, at the pink-cluster stage, corresponding to 15–20% bloom. Further applications were made on 3rd April, at 60% bloom, and on 5th April, at 80% bloom. Also, bromuconazole (0.08% v/v) and difenoconazole (0.02% v/v) were each sprayed seven times; three applications of each fungicide were sprayed on the first three dates, and subsequent applications on 7th April (beginning of petal fall), 9th, 11th and 14th April (Table 1). Untreated trees served as controls.

Effects of fungicides on *A. alternata* colonization of apple fruits at various intervals after full bloom

Ten fruits were randomly collected from each replicate plot and treatment and brought to the laboratory 35, 78, and 97 days after full bloom. Twenty fruits were collected on the harvest date, i.e., 132 days after full bloom (Table 2). In Trial 1—2004, fruits were collected from the following treatments: untreated control, bromuconazole (0.08% v/v), and a tank mixture of bromuconazole (0.08% v/v) with captan (0.25% v/v). Each fruit was cut in half longitudinally and the mesoderm tissue outside the core region of each half was visually examined for the presence of fungal decay and *A. alternata* colonization as described below.

Inoculation of bloom clusters of treated and untreated trees with *A. alternata*

A single-spore culture of *A. alternata* isolate was maintained on PDA plates for 10–12 days until conidia were produced. Conidia were harvested

Table 1 The effects of bromuconazole and difenoconazole, alone and with captan on control of moldy-core in apple fruit in 2004

Treatment and concentration (% v/v)	% of fruits infected with moldy-core[c]
Control	8.0 a
Bromuconazole 0.08 (3 sprays)[a]	4.0 b
Bromuconazole 0.08 + captan 0.25 (3 sprays)[a]	2.0 c
Difenoconazole 0.02 (3 sprays)[a]	5.0 ab
Difenoconazole 0.02 + captan 0.25 (3 sprays)[a]	1.0 c
Bromuconazole 0.08 (7 sprays)[b]	6.0 ab
Difenoconazole 0.02 (7 sprays)[b]	5.0 ab

[a] Three foliar sprays of each fungicide or of a tank mixture were applied in 2004 on: 31st March, at the pink-cluster stage (15–20% bloom), 3rd April (60% bloom) and 5th April (80% bloom)

[b] Bromuconazole (0.08% v/v) and difenoconazole (0.02% v/v) were each sprayed seven times. Three applications of each fungicide were sprayed on the above three dates, and subsequent applications on 7th April (beginning of petal fall), 9th, 11th and 14th April Untreated trees served as controls

[c] Twenty fruits from each replicate were examined for the presence of moldy-core one day before harvest. Means within columns followed by different letters are significantly ($P = 0.05$) different according to the Waller–Duncan K-ratio t-test

from PDA as described above, and the spore concentration was adjusted with the aid of a heamocytometer to give a suspension containing 5×10^5 spores ml^{-1}. The conidial suspension was held on ice and was used to spray-inoculate bloom clusters at full bloom, in the orchard. In the late afternoon of 4th April the conidial suspension was sprayed, at about 6 ml per shoot, onto 20 shoots that contained bloom clusters (70% bloom) in each of five replicates of three selected treatments (Table 3). Inoculated shoots of different treatments were labelled accordingly. To maintain high humidity following spray inoculation, the shoots were covered with wet plastic bags, which were removed the following morning. Control shoots were sprayed with distilled water.

Efficacy of fungicides—Experiment 2

The 2005 experiment was conducted in the same orchard and comprised six treatments as follows: untreated control; difenoconazole at 0.02% v/v, alone and tank mixed with captan at 0.25% v/v; bromuconazole at 0.08% v/v, alone and tank mixed with captan at 0.25% v/v; and captan at 0.25% v/v. Each treatment was applied three times to 16 year-old 'Oregon Spur' trees on 'Hashabi' local rootstock, the first application being at the pink-cluster (10% bloom) stage on 9th April, 2005, and subsequent applications on 11th April (60–70% bloom) and 13th April (full bloom and beginning of petal fall) (Table 4). Untreated trees served as controls. The treatments were arranged as described above and the procedure to determine the percentages of fruits infected with moldy-core is described below.

Large scale demonstration trials in commercial orchards

To further evaluate the potential use of a tank mixture of 0.25% (v/v) captan with either bromuconazole at 0.08% (v/v) or difenoconazole at 0.02% (v/v) against

Table 2 Time study of colonization of *A. alternata* in fruits of fungicide-treated and untreated apple trees

Treatment and concentration (% v/v)[a]	% of fruits colonized by *A. alternata* at various days after full bloom[b]			
	35	78	97	132 (harvest)
Control	7.0 a	26.0 a	36.0 a	58.0 a
Bromuconazole 0.08[a]	0.0 a	0.0 b	20.0 b	37.0 b
Bromuconazole 0.08 + captan 0.25[a]	0.0 a	0.0 b	16.0 b	22.0 c

[a] Three foliar sprays of bromuconazole or of a tank mixture with captan were applied in 2004 on the same dates as given in Table 1

[b] Ten fruits from each replicate and treatment (Table 1—2004 trial) were examined for the presence of *A. alternata* at 35, 78 and 97 days after full bloom and 20 fruits were examined at harvest. Means within columns followed by different letters are significantly ($P = 0.05$) different according to the Waller–Duncan K-ratio t-test

Table 3 The effects of *A. alternata* inoculation at full bloom, of trees that had been sprayed with bromuconazole alone and with captan, on control of moldy-core in apple fruits in 2004

Treatment and concentration (% v/v)	% of fruits infected with moldy-core[b]	% of fruits colonized by *A. alternata*[b]
Control water sprayed	20.0 ab	50.6 ab
Control inoculated	31.0 a	65.0 a
Bromuconazole 0.08[a]	18.0 ab	31.0 bc
Bromuconazole 0.08 + captan 0.25[a]	6.0 b	22.0 c

[a] Foliar sprays of each fungicide or of a tank mixture were applied in 2004 on: 31st March, at the pink-cluster stage (15–20% bloom); 3rd April (60% bloom); and 5th April (80% bloom)

Bloom clusters were inoculated on 4th April (70% bloom). As a control, shoots were sprayed with distilled water

[b] Twenty fruits from each replicate were visually examined for the presence of moldy-core and of *A. alternata* in fruits one day before harvest. Means within columns followed by different letters are significantly ($P = 0.05$) different according to the Waller–Duncan *K*-ratio *t*-test

Table 4 The effect of bromuconazole and difenoconazole, alone and with captan on control of moldy-core in apple fruit in 2005

Treatment and concentration (% v/v)	% of fruits infected with moldy-core[b]
Control	7.0 a
Bromuconazole 0.08[a]	2.0 b
Captan 0.25[a]	9.0 a
Bromuconazole 0.08 + captan 0.25[a]	2.0 b
Difenoconazole 0.02[a]	9.0 a
Difenoconazole 0.02 + captan 0.25[a]	3.0 b

[a] Each treatment was applied in 2005 on: 9th April, at the pink-cluster (10% bloom) stage; 11th April (60–70% bloom); and 13th April (full bloom and beginning of petal fall). Untreated trees served as controls

[b] Twenty fruits from each replicate were examined for the presence of moldy-core one day before harvest. Means within columns followed by different letters are significantly ($P = 0.05$) different according to the Waller–Duncan *K*-ratio *t*-test

moldy-core, large-scale demonstration trials were conducted in 2005 in commercial orchards in the Golan Heights and Upper Galilee regions. Three trials were conducted: two in Ein Zivan orchard and one in Baram orchard in the upper Galilee. Unless stated otherwise, the trials included the following five treatments: untreated control, bromuconazole at 0.08% (v/v), difenoconazole at 0.02% (v/v), and tank mixtures of each fungicide with captan at 0.25% (v/v) (Table 5). The fungicides and mixtures were sprayed three times, at 1,500 l ha^{-1}, with a 2,000 l speeder-type sprayer. The sprays were applied at the beginning of bloom (10%) on 10th April 2005, and on 10th and 14th April, at 60% bloom and beginning of petal fall, respectively. The fungicides were applied to about 0.5 ha of 'Top Red' and 'Oregon Spur' trees, aged 14 and 18 years, respectively, growing on MM.106 rootstock. These trees were in the Ein Zivan orchard, spaced at 2×4 m, with two rows of different cultivars as buffers between treatments. In the Baram orchard applications were made on 12th, 14th and 17th April 2005, at 15–20%, 50%, and full bloom, respectively. Five untreated trees at the end of each row were used as controls.

Assessment of infected fruits with moldy-core

One day before harvest, 20 fruits were randomly collected from each replicate plot and were brought to the laboratory. From the large-scale demonstration trials, 100 fruits were randomly collected from each treatment. Each fruit was cut in half longitudinally and the mesoderm tissue outside the core region of each half was visually examined for the presence of fungal decay. The percentages of fruits in which the mesoderm (flesh tissue) was infected with moldy-core were determined.

In the experiment in which apple trees at full bloom were inoculated with *A. alternata*, two fruits were collected from each of 20 shoots in each replicate plot and treatment. Each fruit was cut in half longitudinally and visually examined as described above. In addition, when colonization of *A. alternata* was observed, a small part of the mesoderm tissue, located about 2 mm outside the core region, was aseptically removed from each half of each fruit and plated on PDA in a sterile 9 cm Petri dish. Samples for this purpose were taken from the following three treatments of the 2004

Table 5 The effects of bromuconazole and difenoconazole alone and with captan on control of moldy-core in apple fruit in large-scale demonstration trials, 2005

Treatment and concentration (% v/v)	% of fruits infected with moldy-core[b] (Mean ± S.E.)		
	Trial 1 Baram Top Red	Trial 2 Ein Zivan Top Red	Trial 3 Ein Zivan Oregon
Control	7.0 ± 0.6	4.0 ± 0.3	7.0 ± 0.4
Bromuconazole 0.08[a]	4.0 ± 0.4	2.0 ± 0.2	6.0 ± 0.5
Bromuconazole 0.08 + captan 0.25[a]	2.0 ± 0.2	0.0 ± 0.0	2.0 ± 0.2
Difenoconazole 0.02[a]	5.0 ± 0.2	2.0 ± 0.3	Not tested
Difenoconazole 0.02 + captan 0.25[a]	3.0 ± 0.1	1.0 ± 0.2	Not tested

[a] Each treatment was applied three times to about 0.5–1.0 ha of each orchard, at 10%, 60% bloom and at beginning of petal fall. Five untreated trees in the end of each row served as controls

[b] One hundred fruits from each treatment were examined for the presence of moldy-core one day before harvest

experiment: control untreated, bromuconazole at 0.08% (v/v), and tank mixtures of bromuconazole with captan at 0.25% (v/v) (Table 2). Samples were also taken from the four treatments of the 2004 experiment: water-sprayed control; control inoculated with *A. alternata*; bromuconazole-treated and *A. alternata*-inoculated; and treated with the bromuconazole/captan mixture and *A. alternata*-inoculated (Table 3). The plates were incubated at 25°C in the dark for 8–10 days; colonies and conidia of *A. alternata* were then identified microscopically. The percentages of fruits colonized by *A. alternata* were determined.

Data analysis

Each laboratory experiment was conducted at least twice. Data from repeated experiments were combined for analysis when the variance between experiments was homogeneous. All data were processed with the SAS GLM statistical software package (SAS Institute 1992). For the field experiment, analysis of variance (ANOVA) was applied to arc-sine transformed data. The Waller–Duncan *K*-ratio *t*-test was used to determine whether differences between treatments were significant.

Results

Decay development on detached fruits

Inoculation of mature detached fruits with conidial suspensions of *A. alternata* mixed with aqueous mixtures of bromuconazole at various concentrations revealed that this fungicide was highly effective. A significant inhibition in decay formation by *A. alternata* was seen at a concentration of 0.5 μg ml^{-1} and at 50 μg ml^{-1} decay formation on fruits was completely inhibited (Fig. 1). Inoculation of fruits with *A. alternata* together with an aqueous mixture of bromuconazole and captan showed that this mixture was significantly more effective than either fungicide alone; it completely inhibited decay formation (Fig. 2). Captan, at 1,250 μg ml^{-1}, i.e. the same concentration that was applied in the field, significantly inhibited decay formation, as compared with the control, but did not provide complete inhibition.

Efficacy of fungicides in the field

In 2004, three foliar applications of bromuconazole or difenoconazole reduced the number of fruits that were naturally infected with moldy-core, as determined just prior to commercial harvest, by 50 and 37.5%, respectively, compared with untreated trees (Table 1). However, tank mixtures of each fungicide with captan significantly ($P < 0.05$) improved moldy-core control and reduced the numbers of infected fruits by 75 and 87.5% respectively, compared with untreated trees (Table 1). There were no significant differences between three and seven foliar applications of either bromuconazole or difenoconazole, in reducing the percentages of infected fruits. A similar trend was seen in a time study in which the percentages of fruits colonized by *A. alternata* were examined (Table 2). Fruits of trees treated with a tank mixture of bromuconazole and captan were the least colonized with *A. alternata* at various days after full

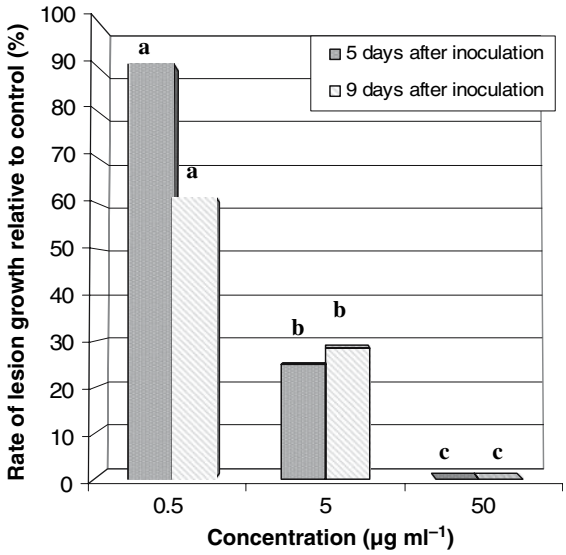

Fig. 1 Effect of bromuconazole on decay development on detached apple fruits. The decay lesion diameter at each inoculation site was measured every 2–3 days; rate of lesion growth on days 5 and 9 after inoculation relative to the control are presented. Three fruits were used for each concentration (µg a.i. ml^{-1}). Mean values followed by different letters are significantly (P = 0.05) different according to the Waller–Duncan K-ratio t-test

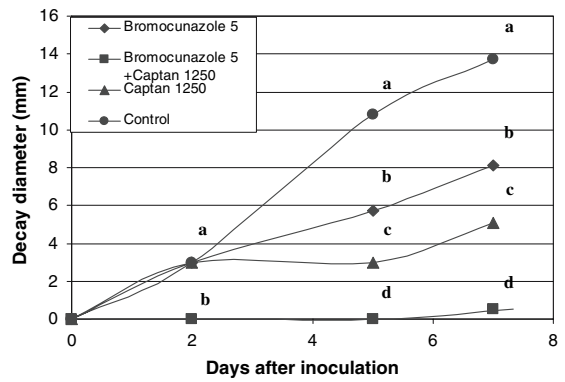

Fig. 2 Effects of bromuconazole, captan and their mixture on decay development on detached apple fruits. The lesion diameter of the decay at each inoculation site was measured every 2–3 days. Three fruits were used for each treatment. Concentrations of each fungicide or their mixture are given as µg a.i. ml^{-1}. Mean values followed by different letters are significantly (P = 0.05) different according to the Waller–Duncan K-ratio t-test

bloom, followed by those treated with bromuconazole alone and those of control untreated trees (Table 2). The enhanced efficacy of a tank mixture with captan was also demonstrated following *A. alternata* inoculations in the orchard, of bloom clusters of treated and of untreated trees at full bloom (Table 3): the greatest protection of the fruits against moldy-core was provided by a bromuconazole/captan mixture, and the percentages of infected and *A. alternata*-colonized fruits in this treatment were significantly ($P < 0.05$) lower than in the control.

Similar results were obtained in 2005. Three applications of a tank mixture of captan with either bromuconazole or difenoconazole gave better control of moldy-core than either difenoconazole or captan alone, but no better than bromuconazole alone (Table 4). Foliar applications of difenoconazole or captan alone were ineffective, and disease incidence was similar to that in untreated control trees.

The results of three large-scale demonstration trials in commercial orchards during 2005 supported those obtained in experimental plots. Foliar applications of a tank-mixture of either bromuconazole plus captan or difenoconazole plus captan reduced the percentage of moldy-core-infected fruits from 4–7% in the untreated controls to 0–3% in the treated fruits (Table 5).

Discussion

Attempts to control *Alternaria* and moldy-core by using foliar sprays of several fungicides, e.g. benomyl, captan, dodine, iprodione or mancozeb have been unsuccessful in the past (Ellis and Barrat 1983; Combrink et al. 1985; Biggs et al. 1993), probably due to low efficacy. The present study showed that either bromuconazole or difenoconazole effectively controlled moldy-core when applied as tank mixes with captan. This was true both for detached apples artificially inoculated in the laboratory and treated with bromuconazole and captan, and for naturally infected trees that were sprayed with each of the fungicides and mixtures. Three foliar applications of each mixture, between the beginning of bloom and petal fall, reduced the numbers of fruits infected with moldy-core by up to 87%, compared with those on untreated control trees. A previous study, which involved artificial inoculations in the orchard, showed that the beginning of bloom and full bloom were the developmental stages most susceptible to infection by the pathogen (Reuveni et al. 2002). These findings may encourage the use of these

mixtures as alternatives to difenoconazole or bromuconazole alone for controlling moldy-core (Reuveni et al. 2002; Reuveni 2006). The seasonal increase in percentages of fruits colonized by *A. alternaria* (Table 2) while infection occurs during bloom period could be related to changes in environmental conditions (e.g. increase in temperature) or to physiological and/or biochemical changes in the inner parts of the fruits. It could also be related to the inoculum level which could be detected at various growth stages of the fruit.

Fungicides are combined in mixtures mainly to widen the spectrum of antifungal activity and to extend their duration; to exploit the synergistic interaction between the compounds, whereby the overall activity can be increased or the amounts used can be reduced without loss of activity; and to delay or reduce the emergence of resistant strains. Synergy, which frequently occurs in fungicide mixtures, may involve antifungal compounds of different natures and sources, fungicides with differing or identical modes of action, and those prepared in different formulations (Gisi 1996). The enhanced efficacy of DMI/captan mixtures, at least that of the difenoconazole mixture (Table 4), compared with their individual efficacy, suggests a synergistic interaction. As the synergy factor (SF), based on Abbott (Kossman and Cohen 1996), was around 1.9, i.e. >1.0, the interaction was, according to Gisi (1996), synergistic. In general, when SF values are greater than 1.5, the interaction may be considered to be strong. In the light of the present findings, the tank mix treatment is being considered for recommendation as a strategy for the control of moldy-core on apple trees in the northern region of Israel.

The present authors and co-workers (Reuveni et al. 2002, Reuveni 2006) recently showed that difenoconazole and bromuconazole can be effective alternative fungicides for control of *Alternaria* moldy-core in apple fruits. The mode of action of captan is different from those of sterol inhibitor fungicides (Koller and Scheinpflug 1987). Captan is a multi-site contact phthalimide fungicide that is thought to inhibit spore germination. Our present findings suggest that captan was ineffective against *Alternaria* and moldy-core when applied alone in the field, but that it provided improved control when applied together with a DMI fungicide.

The mode of action of the sterol biosynthesis inhibitors, including bromuconazole and difenoconazole, has been extensively investigated (Koller and Scheinpflug 1987). They are inhibitors of the C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol, a biosynthesis step that occurs during the conversion of lanosterol to ergosterol, the final product of fungal cell wall sterol synthesis (Koller and Scheinpflug 1987).

As captan and DMI fungicides have different modes of action (Koller and Scheinpflug 1987; Gisi et al. 2000), their use in combination could be incorporated into a disease management programme that would minimize the risk of resistance development by *Alternaria* spp. and at the same time, maximize disease control. The activities of tank mixes of captan with bromuconazole or difenoconazole on *A. alternata* and on postharvest decay formation in apple fruits, and their efficacy in the field, indicate that these mixtures could potentially control moldy-core disease.

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