

Colonization of Table Grape Bunches by *Alternaria alternata* and Rot of Cold-Stored Grapes

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Submitted for publication: May 1994

Accepted for publication: September 1994

Key words: Disease control, endophyte, latent infection, postharvest decay, stress factors, wound pathogen

Assessment of latent *Alternaria alternata* infections in table grapes indicated that infections occurred in commercial vineyards during the entire period of bunch development. Mature bunches were asymptomatic despite high levels of *A. alternata* recovered from triple-sterilized bunch tissue. Inoculation studies showed no shift in disease susceptibility of ripening grape berries, and postharvest rot was not related to the level of natural infection. Late season fungicidal sprays, or dip treatments that ensured better penetration and coverage of inner parts, resulted in no meaningful reduction in postharvest rot. Based on the behaviour of the pathogen, it is suggested that additional fungicide programmes for the control of the disease should not be followed in commercial vineyards. Instead, attention should be given to physiological and stress factors, such as mechanical and sulphur dioxide damage that might predispose cold-stored bunches to *A. alternata* decay.

Alternaria alternata (Fries) Keissler causes rot of stored table grapes (Harvey, 1955), particularly those in cold storage (Swart & Holz, 1991). The disease is characterized by fairly firm, superficial lesions which often occur on berries near the pedicel. Lesions are tan at first, become dark brown to black with age and remain localized. Under humid conditions provided by cold transit, fluffy gray tufts of fungus often occur on rachi and pedicels, occasionally without causing visible lesions (Swart & Holz, 1991). Most of South Africa's table grape production, nearly 15.5 million cartons per year is exported (D.P. Hugo, Unifruco, P.O. Box 505, Bellville 7535, personal communication). Annual losses due to *Alternaria* rot are usually negligible (Combrink & Truter, 1979; Swart & Holz, 1991). However, an analysis of data from individual producers indicates that the disease occurs sporadically and is usually confined to specific consignments. In such cases high incidences of bunches with *A. alternata* growth were occasionally recorded (Swart & Holz, 1991). The disease is therefore of great concern to the local table grape industry.

Little is known about the environmental and physiological criteria for *A. alternata* fruit rot development on table grapes. Table grapes for export are routinely fumigated with sulphur dioxide (SO₂) during shipment to protect them against *Botrytis cinerea* infection (László *et al.*, 1981; Nelson, 1983). Fumigation with SO₂ kills *A. alternata* and *B. cinerea* spores or mycelium on the surface of grapes, but does not kill mycelium that has invaded the berry (Couey, 1965; Smilanick *et al.*, 1990). Symptoms of *Alternaria* bunch rot on export grapes are only evident at the end of prolonged cold storage (Swart & Holz, 1991). Latent field infections undetectable at harvest may therefore play a prominent role in postharvest rot. Similar infections by *A. alternata* are commonly found in mango (Prusky *et al.*, 1983), persimmon (Prusky *et al.*, 1981), citrus fruit (Eckert, 1975) and papaya (Alvarez *et al.*, 1977).

Susceptibility of mango (Prusky *et al.*, 1983), persimmon (Prusky *et al.*, 1981) and New Mexican-type chile peppers (Wall & Biles, 1993) to *A. alternata* rot is related to changes as fruit ripens. A similar relationship has been reported for *B. cinerea* on grapes, where berries become more susceptible to bunch rot after véraison (Bulit & Dubos, 1988). Our objectives were to detect the presence of *A. alternata* in table grapes grown in commercial vineyards at particular developmental stages and post-storage and to determine the relative susceptibility of table grapes bunches to *A. alternata* rot as fruit develops and matures. In addition several fungicides were evaluated for their efficacy against the pathogen.

MATERIALS AND METHODS

Colonization of grape bunches by *A. alternata*: The studies were conducted in commercial vineyards. All vines were trained to a slanting trellis and were micro-irrigated. Different programmes for the control of downy and powdery mildew and *Botrytis cinerea* bunch rot were followed in commercial vineyards. Sprays against downy mildew started at 10-15 cm shoot length and were applied every 14 days with mistblowers until pea size. Fungicides used were folpet (Folpan 50% WP, Agrihold), fosetyl-Al/mancozeb (Mikal M 44/26% WP, Maybaker), mancozeb (Dithane M45 80% WP, FBC Holdings) and mancozeb/oxadixyl (Recoil 56/8% WP, Bayer). Applications against powdery mildew started at 2-5 cm shoot length and were applied every 14 days with mistblowers until 3 weeks before harvest. Fungicides used were penconazole (Topaz 10% EC, Ciba-Geigy), pyrifenoxy (Dorado 48% EC, Maybaker) and triadimenol (Bayfidan 25% EC, Bayer). Four to eight applications against *Botrytis cinerea* were applied from full bloom until one week before harvest. Fungicides used were iprodione (Rovral 25% SC, Maybaker; Astryl 20% EC, Maybaker), iprodione/sulphur (Rovral/sulphur 3/90% DP, Maybaker) and procymidone (Sumisclax 25% SC, Agricura).

During the 1987/88 season, two vineyards with cultivars Dan-ben-Hannah and Queen of the Vineyard were selected in the Paarl region. Each vineyard was divided into nine plots. Five bunches (total of 45 bunches/sampling) were sampled at random from each plot during the season and post-storage (Fig. 1). At each sampling, bunches were examined for symptoms of *Alternaria* rot, surface sterilized (70% ethanol for 5 sec, 1 min in 0.35% NaOCl followed by 5 sec in 70% ethanol) and dried on a laminar flow bench. Ten pieces each of rachis, pedicels, berry-bases and stylar-ends were cut aseptically from bunches. Tissue lengths from rachis and pedicels were 1 cm, whereas tissue discs from berries were 8 mm². The tissue discs were placed on corn-meal agar (CMA) supplemented with thiabendazole (20 µg/ml) (Prusky *et al.*, 1983) in petri dishes and incubated at 25°C for 10 days. Developing colonies were examined with a light microscope (10X). *Alternaria* spp. were transferred to half-strength malt agar or standard potato-carrot agar plates, incubated at 25°C under a mixture of near-ultra violet and cool fluorescent light, and identified according to the criteria of Simmons (1967). Tissue discs yielding *A. alternata* were then recorded.

The survey was repeated during the 1988/1989 season in the Dan-ben-Hannah vineyard. Two Waltham Cross vineyards were also included in the survey. Waltham Cross I was pruned earlier and therefore bloomed 2 weeks earlier than Waltham Cross II. The two Waltham Cross vineyards were divided into 30 plots each. Bunches were collected every 2 weeks (Fig. 2) to obtain a more even distribution of colonization throughout the season, and post-storage. Isolations were made from surface sterilized bunches as previously described.

Relation between tissue colonization and *Alternaria* post-harvest rot: The relation between tissue discs yielding *A. alternata* at harvest and *Alternaria* postharvest rot was determined with fruit from the commercial vineyards. Bunches were obtained from the vines used for the *A. alternata* monitor studies and packed as for export with an SO₂ generator (0.3-0.55 g sodium metabisulfite affixed to a paper sheet [László *et al.*, 1981; Nelson, 1983]) inside a polyethylene bag in corrugated boxes (Patent no. RSA 75/6116). During the 1987/88 season, six boxes were selected from each vineyard. During the following season, 90 boxes of Dan-ben-Hannah and 120 boxes from each Waltham Cross vineyard were selected. Grapes were stored at -0.5°C for 3 weeks followed by 1 week at 10°C to simulate shipment. Grapes were then transferred to 25°C for an additional 5-day period to promote disease development. Infected berries were removed from the bunches and the percentage *Alternaria* berry rot in each bunch was calculated on a mass basis. *Alternaria* growth on the rachis was assessed according to the evaluation rating proposed by Unterstenhöfer (1963) for the infection of berries by *Plasmopara viticola*, and the percentage growth on each bunch calculated with the formula of Kremer & Unterstenhöfer (1967). To evaluate the effect of SO₂ fumigation on *A. alternata*, samples were collected from bunches and isolations were made as previously described.

Inoculation studies: The ability of *A. alternata* to infect and cause decay of Waltham Cross grapes at different stages of maturity was determined in moist chambers in the laboratory. A pathogenic isolate of *A. alternata*, isolated from a grape berry (Swart & Holz, 1991), was maintained in a freeze-dried state. Inoculum was prepared by growing the fungus for 14 days on potato-dextrose agar (PDA) in petri dishes at 25°C under a mixture of near-ultra violet and cool fluorescent light. The cultures were flooded with sterile distilled water containing 0.1% Tween 20, the conidia dislodged with a sterile glass rod and the spore suspension filtered through two layers of cheesecloth. Spore counts were made with a haemocytometer and the suspensions adjusted to approximately 1.5 x 10⁶ spores/ml.

Sound, unblemished bunches were obtained from the Waltham Cross II vineyard at pea size, véraison and at harvest. The bunches were surface sterilized as previously described and dried on a laminar flow bench. To inoculate the different bunch parts, some of the berries including pedicels were removed from each bunch to expose the rachis. Bunches were then placed on top of water-saturated "oases" (florist's sponge) covered with sterile aluminium-foil, and their stems plugged into small holes made into the sponge. The oases were placed in sterile distilled water in moist chambers at 25°C. This ensured the high humidity needed for infection (Hewitt, 1974). Droplets (20 µl) containing approximately 200 spores were placed on rachis, pedicels or berries with a Pasteur pipette. Inoculated parts were examined daily for lesion development.

In a separate experiment, bunches obtained at harvest from the same vineyard were surface sterilized and dried. They were packed in polyethylene bags in boxes and sprayed with inoculum, avoiding runoff. The bags were sealed, but SO₂ generators were not included. Boxes with grapes were stored and evaluated for *Alternaria* postharvest rot as previously described.

Fungicide tests in culture: PDA was amended with 10 µg active ingredient of fungicide per milliliter with the following fungicides: captab, (Kaptan 50% WP, AECI), triadimefon (Bayleton 25% EC, Bayer), benomyl (Benlate 50% WP, Agricura), mancozeb (Dithane M45 80% WP, FBC Holdings), triadimenol (Bayfidan 25% EC, Bayer), folpet (Folpan 50% WP, Agrihold), chlorothalonil (Bravo 50% SC, Shell Chemicals), propineb (Antracol 70% WP, Bayer), vinclozolin (Ronilan 50% SC, BASF), metiram (Polyram Combi 80% WP, BASF), nuarimol (Trimidal 9% EC, FBC Holdings), procymidone (Sumisclec 25% SC, Agricura), penconazole (Topaz 10% EC, Ciba-Geigy), dinocap (Karathane 20% WP, Agrihold), iprodione (Rovral Flo 25% SC, Maybaker Agrichem), chloramizol (Fungaflor 19% EC, Shell Chemicals), hexaconazole (Anvil 5% EC, ICI Agrochemicals), pyri-fenox (Dorado 48% EC, Maybaker), prochloraz (Sportak 45% EC, FBC Holdings), flusilazole (Nustar 40% EC, Agricura) and metalaxyl (Apron 35% WP, Ciba-Geigy). All fungicides were incorporated into the agar at 45°C as suspensions.

For growth experiments 8-mm-diameter mycelial plugs cut from the growing edge of a 10-day-old *A. alternata* colony were inverted on the surface of amended or unamended PDA in petri dishes. Each treatment was replicated seven times. Linear measurements were taken after 7-day incubation at 23°C by measuring two diameters of the growing colony at right angles to one another. The percentage spore germination was determined on amended and unamended water agar at 23°C by scoring 100 spores. Each treatment was replicated three times.

Fungicide tests in the vineyard: Fungicide treatments were tested for two consecutive seasons in the commercial Waltham Cross vineyard. As some of the fungicides applied in the standard programme are known to reduce infection by *A. alternata* on other crops (Profic-Alwasiak & Szczygiel, 1980; Kalra & Sori, 1985; Williams, 1987; Jones *et al.*, 1989), application of these commercially applied fungicides was discontinued approximately 3 weeks before harvest.

During the 1988/89 season, 9 days after termination of the spray programme and 16 days before harvest, unblemished bunches were selected at random on vines in the centre of the vineyard. The bunches were surface sterilized by dipping for 1 min in 0.35% NaOCl. Two experiments were then conducted to evaluate the protective and curative ability of the selected fungicides. In the first experiment, hexaconazole, pyrifenoxy, iprodione, chloramizol and prochloraz were applied with a rucksack spray pump (Hatsuta multipurpose power unit, AM-8 model "Blowmic") to 125 bunches (25/fungicide) at doses recommended for table grapes. Since superficial growth of the fungus on the rachi and pedicels is a prominent feature of the disease (Swart & Holz, 1991), another group of 125 bunches was dipped for 5 sec into the fungicide suspensions (25 bunches/fungicide) to cover the rachi. Treated bunches were inoculated after 7 days by spraying them with a spore suspension of *A. alternata* as previously described. Inoculated bunches were covered with polyethylene bags and sealed with wire ties for 18 h. These bags contained a little water to maintain high humidity during the infection period. Control bunches were sprayed with distilled water and were covered with bags as described above.

Post-infection activity was determined in the second experiment. Bunches were inoculated and covered with polyethylene bags after surface disinfestation. Fungicidal spray and dip treatments were applied 1 day after inoculation at the rates given above. Control treatments received distilled water.

During the following season, commercial fungicide applications were stopped and the bunches inoculated as described previously. Individual bunches were sprayed with hexaconazole, pyrifenoxy, iprodione, chloramizol, prochloraz, captab, folpet, propineb, mancozeb, metalaxyl, metiram, flusilazole, nuarimol and procymidone at doses recommended for table grapes. Applications were made either 1 day before or after inoculation. Each treatment consisted of randomly assigned blocks of six vines.

For both seasons bunches from the different treatments were harvested 3 weeks after application of fungicides. In the 1988/89 season, all the treated bunches were packed, whereas during the following season bunches used for storage were selected at random from treated blocks. The grapes (three replicates of two boxes per treatment) were packed as for export and stored at -0.5°C for 3 weeks followed by 1 week at 10°C to simulate overseas shipment.

Grapes were then transferred to 25°C for an additional 5-day period to promote disease development. Since *Alternaria* lesions on the rachis might be confused with those caused by *B. cinerea*, only *Alternaria* berry rot was determined for each bunch on a mass basis and the average percentage decay for each treatment was calculated.

RESULTS

Colonization of grape bunches by *A. alternata*: The fungus was consistently associated with all surface-sterilized bunches, except those of Dan-ben-Hannah sampled during the 1987/88 season. From the 45 bunches sampled from the latter cultivar at either full bloom or harvest, 26 and 38 were colonized by the fungus, respectively.

The pathogen was isolated more frequently from bunches during the 1988/89 season than during the 1987/88 season, but it colonized grape tissue according to a similar pattern (Figs. 1 and 2). This occurred at relatively low incidences on bunch parts at the end of full bloom, except

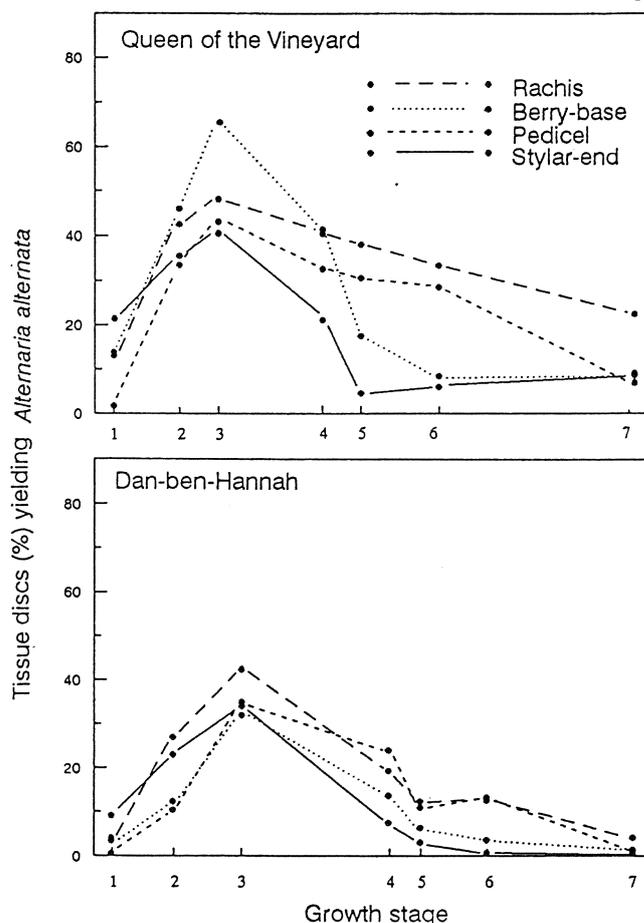


FIGURE 1
Percentage tissue discs, sampled during the 1987/88 season from table grape bunches, yielding *Alternaria alternata* after 2 week incubation on cornmeal agar at 25°C. Sampling periods for cv. Queen of the Vineyard were: 1 = 14/11 (end of full bloom; 100% calyptra-shed); 2 = 25/11 (pea size); 3 = 3/12 (bunch closure); 4 = 22/12 (vérasion); 5 = 29/12; 6 = 12/1 (harvest); 7 = 17/2 (post-storage). Sampling periods for cv. Dan-ben-Hannah were: 1 = 14/11 (end of full bloom; 100% calyptra-shed); 2 = 25/11 (pea size); 3 = 9/12 (bunch closure); 4 = 7/1 (vérasion); 5 = 13/1; 6 = 26/1 (harvest); 7 = 24/2 (post-storage).

on pedicels from which it was infrequently isolated. Incidence levels on all bunch parts increased until bunch closure (1987/88 season, Fig. 1) or around vérasion (1988/89 season, Fig. 2). Although the incidence levels then declined, the pathogen was regularly isolated from picking-ripe bunches. During harvest of the 1987/88 season, *A. alternata* occurred regularly at higher levels on rachi and pedicels than on berry parts (Fig. 1, stage 6). This was not

the case during the 1988/89 season, when higher incidences were generally recorded on berry-base parts (Fig. 2, stage 7 [Dan-ben-Hannah and Waltham Cross I], stage 8 [Waltham Cross II]). Levels of *A. alternata* were reduced by SO₂ fumigation and cold storage (Figs. 1 and 2). An exception was Queen of the Vineyard during the 1987/88 season, which showed a high incidence of *A. alternata* on rachi after cold storage.

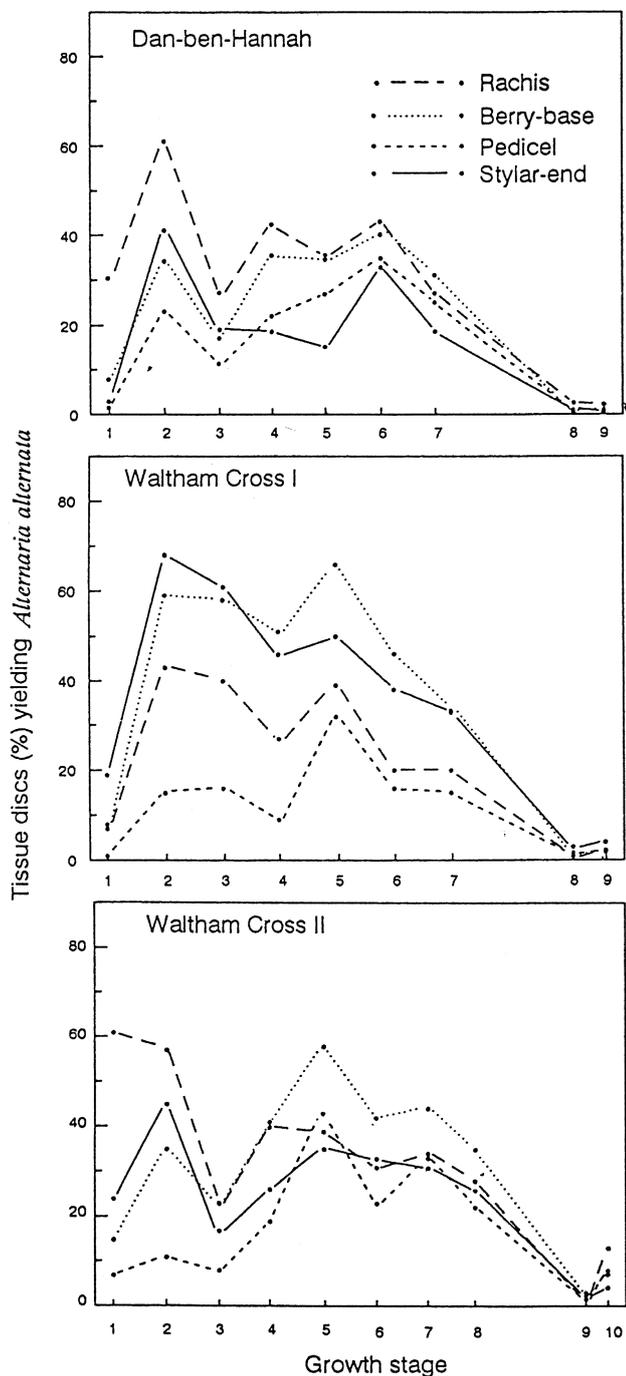


FIGURE 2

Percentage tissue discs, sampled during the 1988/89 season from table grape bunches, yielding *Alternaria alternata* after 2 week incubation on cornmeal agar at 25°C. Sampling periods for cv. Dan-ben-Hannah were: 1 = 2/11 (end of full bloom; 100% calyptra-shed); 2 = 16/11 (pea size); 3 = 30/11 (bunch closure); 4 = 14/12; 5 = 26/12 (véraison); 6 = 11/1; 7 = 25/1 (harvest); 8 = 29/2 (post-storage); 9 = 8/3. Sampling periods for cv. Waltham Cross I were: 1 = 25/10 (end of full bloom; 100% calyptra-shed); 2 = 9/11 (pea size); 3 = 23/11 (bunch closure); 4 = 7/12; 5 = 21/12 (véraison); 6 = 4/1; 7 = 18/1 (harvest); 8 = 17/2 (post-storage); 9 = 25/2. Sampling periods for cv. Waltham Cross II were: 1 = 11/11 (end of full bloom; 100% calyptra-shed); 2 = 25/11 (pea size); 3 = 9/12 (bunch closure); 4 = 22/12; 5 = 6/1; 6 = 20/1 (véraison); 7 = 3/2; 8 = 16/2 (harvest); 9 = 17/3 (post-storage); 10 = 23/3.

In spite of the high incidences of tissue discs yielding *A. alternata* during sampling, *Alternaria* rot was not observed on bunches during any growth stage.

Relation between tissue colonization and *Alternaria* post-harvest rot: No clear relation was found between percentage *Alternaria* rot of cold-stored grapes and the incidence of bunch parts yielding *A. alternata* at harvest (Table 1). For example, berry base and styler-end tissue discs from Dan-ben-Hannah yielded exceptionally high incidences of *A. alternata* during the 1988/89 season, whereas only 0.17% berry rot was recorded. On the other hand, during the 1987/88 season on the same cultivar, low *A. alternata* incidences were recorded on these bunch parts, but 1.12% berry rot was recorded.

Inoculation studies: Final evaluations were made 7 days after inoculation when rachi and pedicels became flaccid. At that stage visible lesions had not formed on any of the inoculated parts.

Bunches sprayed with inoculum and kept under conditions simulating overseas shipment were extensively covered with superficial growth of *A. alternata*. This hampered disease scoring on berries. However, typical *Alternaria* berry rot was observed on individual berries in only a few bunches (data not included).

Fungicide trials: The effect of different fungicides on germination of conidia and on mycelial growth is given in Table 2. Germination was completely inhibited by captab on folpet and drastically reduced by mancozeb, propineb, metalaxyl and metiram. Of these fungicides, metalaxyl and metiram reduced mycelial growth by more than 50%. Mycelial growth was almost stopped by pyrifenoxy, prochloraz and flusilazole, and effectively inhibited ($\geq 70\%$ reduction in growth) by procymidone, penconazole, dinocap, iprodione, chloramizol and hexaconazole.

In spite of efforts taken to promote infection in the vineyard, *A. alternata* rot did not develop during the 1988/89 season on bunches of most treatments stored with an SO₂ sheet, whereas during the following season, the percentage of decay of the fungicide-free bunches (Table 3) was generally less than that of fungicide-treated bunches. Data were therefore not subjected to statistical analysis. However, the data indicated that in these experiments SO₂ reduced *A. alternata* rot.

DISCUSSION

This study confirms the findings of an earlier report (Swart & Holz, 1991) which showed that *Alternaria* post-harvest rot is not caused by a pathotype of *A. alternata*, but by opportunistic forms of the fungus. Some variation may occur in the parasitism of the many strains, but these tests and the unreported ones with other isolates indicated that in general they were unable to cause active rot when inoculated into healthy tissue of green and mature table grape bunches. The consistent isolation of *A. alternata* from rachi, pedicels and berries furthermore provides evidence that colonization results from discrete colonies developing from conidia deposited at random on the bunch during the entire period of growth. Similar continuous infection by *A. alternata* has been found in apricot (Larsen *et al.*, 1980), persimmon (Prusky *et al.*, 1981) and mango (Prusky *et al.*, 1983) during fruit growth. Inoculum can be provided by saprophytic forms of *A. alternata*, which are ubiquitous and colonize many substrates (Domsch *et al.*, 1980), and by pathogenic forms which attack a variety of

TABLE 1

Tissue discs sampled at harvest from grape bunches¹ exhibiting growth of *Alternaria alternata*, and *A. alternata* postharvest decay of grapes after cold storage.

| Season and cultivar | Discs with fungal growth (%) ^{2,3} | | | | Postharvest decay (%) ^{3,4} | |
|-----------------------|---|-----------|------------|------------|--------------------------------------|-----------|
| | Rachis | Pedicele | Berry-base | Stylar-end | Rachis | Berries |
| 1987/88 | | | | | | |
| Dan-ben-Hannah | 13.1(2.2) | 13.3(2.3) | 3.7(1.5) | 0.8(0.4) | 4.52(1.5) | 1.12(0.9) |
| Queen of the Vineyard | 33.5(3.3) | 28.6(2.6) | 8.4(2.1) | 6.2(1.6) | 4.00(1.4) | 0.91(0.5) |
| 1988/89 | | | | | | |
| Dan-ben-Hannah | 27.3(2.7) | 25.3(2.8) | 31.3(2.5) | 18.8(2.1) | 6.00(0.2) | 0.17(0.1) |
| Waltham Cross I | 20.0(2.1) | 16.9(2.6) | 33.0(3.1) | 33.0(3.4) | 2.13(1.0) | 0.14(0.3) |
| Waltham Cross II | 28.0(3.3) | 22.0(3.7) | 35.0(4.2) | 26.0(3.6) | 3.00(1.2) | 0.56(0.2) |

¹ Bunches selected for isolations and cold storage were from the same vineyard.

² Tissue discs incubated on CMA supplemented with thiabendazole (20 µg active ingredient/ml) at 25°C.

³ Figures between brackets indicate standard deviation.

⁴ Bunches packed as for export inside a polyethylene bag with an SO₂ generator; percentage *Alternaria* berry rot in each box determined on a mass basis and the average percentage decay calculated; *A. alternata* growth on the rachi assessed according to the evaluation rating proposed by Unterstenhöfer (1963) and the percentage growth of each bunch calculated with the formula of Kremer & Unterstenhöfer (1967).

hosts (Dickinson, 1981). Colonization of loose floral debris within bunches by the fungus has also been observed (Hewitt, 1974). This debris most probably served as foci for inoculum in closing bunches. A continual showering of bunches with spores of the fungus can therefore be expected to occur.

Verhoeff (1974) described latent infections as a quiescent or dormant parasitic relationship which, after time, can change into an active one. Our data suggest that *A. alternata* does not only form latent infections, but may also occur as an endophyte on table grapes. In modern terminology the term endophyte is used for living organisms detected inside healthy plant tissue (Sieber *et al.*, 1990) and is generally restricted to fungi causing asymptomatic infections entirely within the tissues of plants (Carroll, 1986). The most common method of detection of endophytes involves a triple sterilization of host tissue (Petrini, 1986; Sieber *et al.*, 1990) to eliminate superficial colonizers, and isolation onto laboratory media. We found that mature bunches were asymptomatic despite high levels of *A. alternata* recovered from triple sterilized bunch tissue. There was furthermore no shift in disease susceptibility of ripening grape berries, as was reported for *A. alternata* on other fruit types (Prusky *et al.*, 1981; Prusky *et al.*, 1983; Wall & Biles, 1993). *A. alternata* has been reported as an endophyte of tobacco (Delabays & Corbaz, 1987) and mango (Johnson *et al.*, 1992).

A. alternata is generally considered as a weak pathogen that infects fruit after mechanical damage, sunburn or chilling injury (McColloch & Worthington, 1952; Ben-Arie, 1966; Pearson & Hall, 1975; Prusky *et al.*, 1983; Barkai-Golan & Kopeliovitch, 1989). Our studies support this premise on table grapes. During shipment table grapes are fumigated with SO₂ to protect them against new *B. cinerea* infections (László *et al.*, 1981; Nelson, 1983). This process is regulated by adding a sodium metabisulfite generator inside a polyethylene bag in boxes (László *et al.*, 1981; Nelson, 1983). However, several problems are still encountered with SO₂ treatment. Wounds are usually inflicted at harvest, during sorting and packing. The SO₂ can

TABLE 2

Response of *Alternaria alternata* to fungicides on agar media.

| Fungicides ¹ | Germination (%) ² | Radial growth (mm ²) ³ |
|-------------------------|------------------------------|---|
| Control | 94.33 a | 30.71 a |
| Captab | 0.00 i | 29.43 a |
| Triadimefon | 90.67 a | 28.25 a |
| Benomyl | 89.67 ab | 25.00 b |
| Mancozeb | 17.33 h | 22.17 c |
| Triadimenol | 80.67 de | 22.00 cd |
| Folpet | 0.67 i | 21.75 c |
| Chlorothalonil | 63.33 g | 19.00 d |
| Propineb | 3.33 i | 14.43 e |
| Metalaxyl | 19.33 h | 12.86 ef |
| Vinclozolin | 82.33 cd | 12.83 efg |
| Metiram | 18.33 h | 11.00 fgh |
| Nuarimol | 91.00 a | 10.57 gh |
| Procymidone | 74.33 ef | 9.00 hi |
| Penconazole | 83.67 bcd | 8.17 i |
| Dinocap | 80.00 de | 7.57 i |
| Iprodione | 66.67 g | 7.17 i |
| Chloramizol | 89.67 ab | 4.67 j |
| Hexaconazole | 89.00 abc | 4.00 j |
| Pyrifenoxy | 89.33 ab | 1.67 k |
| Prochloraz | 87.67 bcd | 1.00 k |
| Flusilazole | 68.00 fg | 0.43 k |

¹ Fungicides (10 µg active ingredient/ml) were incorporated into the agar media at 45°C as suspensions.

² Mean of conidia germinated (3 replications) after 24 h on WA at 23°C; values followed by the same letter are not significantly different at the 1% level.

³ Mean colony diameters (7 replications) after 7 days growth on PDA at 23°C; values followed by the same letter are not significantly different at the 1% level.

damage the fruit by entering wounds and openings in their surfaces caused by stem tears and cracks (Ballinger & Nesbitt, 1984). SO₂ can also damage berries by bleaching and can increase the rate of water loss and thus cause premature browning of stems. These effects are usually noticed during storage at -0,5°C on grapes not properly

TABLE 3

Effect of fungicide sprays, applied before harvest, on *Alternaria alternata* postharvest berry rot of table grapes packed with and without an SO₂ sheet.

| Fungicides ² | Berry rot (%) ¹ | | | |
|-------------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|
| | +SO ₂ sheet | | -SO ₂ sheet | |
| | Before inoculation ³ | After inoculation ⁴ | Before inoculation ³ | After inoculation ⁴ |
| Pyrifenox | 0.30 | 0.19 | 2.17 | 2.09 |
| Prochloraz | 0.22 | 0.09 | 1.12 | 1.19 |
| Hexaconazole | 0.00 | 0.07 | 2.67 | 1.70 |
| Flusilazole | 0.46 | 0.46 | 4.27 | 2.45 |
| Nuarimol | 0.05 | 0.22 | 2.45 | 1.61 |
| Iprodione | 0.27 | 0.35 | 0.85 | 1.48 |
| Folpet | 0.41 | 0.53 | 1.96 | 1.82 |
| Captab | 0.06 | 0.27 | 1.18 | 0.69 |
| Mancozeb | 0.38 | 0.21 | 3.33 | 1.20 |
| Propineb | 0.53 | 0.19 | 3.70 | 1.62 |
| Metalaxyl | 0.70 | 0.00 | 2.90 | 1.81 |
| Metiram | 0.05 | 0.36 | 2.32 | 1.21 |
| Procymidone | 0.30 | 0.09 | 2.23 | 3.16 |
| Chloramizol | 0.29 | 0.66 | 4.08 | 1.95 |
| Control | 0.09 | 0.46 | 1.28 | 1.11 |

¹ Percentage *Alternaria* berry rot in each box determined on a mass basis and the average percentage decay calculated.

² Fungicide doses (in 100 l water): pyrifenoX 12 ml; prochlorax 30 ml; hexaconazole 30 ml; flusilazole 50 ml; nuarimol 15 ml; iprodione 200 ml; folpet 200 ml; captab 200 g; mancozeb 350 ml; propineb 200 g; metalaxyl 270 g; metiram 200 g; procymidone 200 ml; chloramizol 50 ml.

³ Fungicides applied 1 day before inoculation; harvest 2 weeks after fungicide treatment.

⁴ Fungicides applied 1 day after inoculation; harvest 2 weeks after fungicide treatment.

pre-cooled (László *et al.*, 1981). Our observation (Swart & Holz, 1991) that superficial growth of *A. alternata* occurred predominantly on flaccid or dry rachis and pedicels, and that lesions were first noticeable during storage at 10°C or higher temperatures, indicated predisposition by SO₂ in some consignments of export grapes. Furthermore, grapes might be weakened by prolonged storage at low temperatures which might cause them to become susceptible to *Alternaria* decay, as was found for tomatoes (McColloch & Worthington, 1952).

Protective treatments, starting after fruit set, are effective in preventing infection by *A. alternata* on papaya (Alvarez *et al.*, 1977), fig (Bewaji *et al.*, 1977) and mango (Prusky *et al.*, 1983). Harvey (1955) found that the application of protective fungicides in the vineyard gave good control of *Alternaria* decay in cold-stored grapes. Mangiarotti *et al.* (1987) showed that *Alternaria* on the grape phylloplane was not particularly influenced by fungicide treatments, while others, such as *Botrytis* and *Epicoccum*, were notably reduced. In this study continual infection of *A. alternata* occurred while grapes were on the vines, in spite of rigorous fungicidal sprays with chemicals that inhibited fungal development *in vitro*. Late season fungicide spray or dip treatments that ensured better penetration and coverage of inner parts also caused no reduction in postharvest decay. Based on the behaviour of the pathogen, an additional fungicide programme would be of no benefit and should not be followed in local vineyards for the control of *A. alternata* rot on cold-stored table grapes. Instead, attention should be given to physiological and stress factors, such as wounding and SO₂ damage that might predispose cold-stored bunches to *A. Alternata* decay.

LITERATURE CITED

- ALVAREZ, A.M., HYLIN, J.W. & OGATA, J.N., 1977. Postharvest diseases of papaya reduced by biweekly orchard sprays. *Plant Dis. Rep.* **61**, 731-735.
- BALLINGER, W.E. & NESBITT, W.B., 1984. Quality of *Euveitis* hybrid bunch grapes after low temperature storage with sulfur dioxide generators. *J. Amer. Soc. Hort. Sci.* **109**, 831-834.
- BARKAI-GOLAN, R. & KOPELIOVITCH, E. 1989. Effect of peel injury and enzymatic activity of the fruit on the tolerance of tomato genotypes to *Alternaria* infection. *Acta Hort.* **258**, 631-637.
- BEN-ARIE, R., 1966. The relationship of wounding and inoculation of Grand Alexander apples to the development of storage decay caused by *Alternaria tenuis* Nees. *Israel J. Agric. Res.* **16**, 179-180.
- BEWAJI, O., ENGLISH, H. & SCHICK, F.J., 1977. Control of *Alternaria* surface rot of Kadota figs. *Plant Dis. Rep.* **61**, 351-355.
- BULIT, J. & DUBOS, B., 1988. *Botrytis* bunch rot and blight. In: PEARSON, R.C. & GOHEEN, A.C. (eds) Compendium of Grape Diseases. The American Phytopathological Society, St Paul, Minnesota. pp. 13-15.
- CARROLL, G.C., 1986. The biology of endophytism in plants with particular reference to woody perennials. In: FOKKEMA, N.J. & VAN DEN HEUVEL, J. (eds) Microbiology of the Phyllosphere. Cambridge University Press, Cambridge. pp. 205-222.
- COMBRINCK, J.C. & TRUTER, A.B., 1979. The control of post-harvest decay of table grapes – a review of developments in South Africa. *Decid. Fruit Grower* **29**, 272-283.
- COUEY, H.M., 1965. Inhibition of germination of *Alternaria* spores by sulfur dioxide under various moisture conditions. *Phytopathology* **55**, 525-527.
- DELABAYS, N. & CORBAZ, R., 1987. Study of the main parasitic fungi of tobacco seed capsules: *Botrytis cinerea* and *Alternaria alternata*. *Rev. Suisse d'Agric.* **19**, 25-28.
- DICKINSON, C.H., 1981. Leaf surface micro-organisms as pathogen antagonists and as minor pathogens. In: JENKYN, J.F. & PLUMB, R.T. (eds) Strategies for the Control of Cereal Disease. Blackwell Scientific Publications, Oxford. pp. 109-121.
- DOMSCH, K.H., GAMS, W. & ANDERSON, T.H., 1980. Compendium of Soil Fungi. Volume 1. Academic Press, London.

- ECKERT, J.W., 1975. Postharvest pathology. In: PANTASTICO, R.B. (ed.) Postharvest physiology, handling and utilization of tropical and subtropical fruits and vegetables. Avi Publishing Co., Westport. pp. 393-443.
- HARVEY, J.M., 1955. Decay in stored grapes reduced by field applications of fungicides. *Phytopathology* **45**, 137-140.
- HEWITT, W.B., 1974. Rots and bunch rots of grapes. *Calif. Agric. Exp. Stn. Bull.* 868.
- JOHNSON G.I., MEAD, A.J., COOKE, A.W. & DEAN, J.R. 1992. Mango stem end rot pathogens – Fruit infection by endophytic colonisation of the inflorescence and pedicel. *Ann. Appl. Biol.* **120**, 225-234.
- JONES, A.L., EHRET G.L. & COMSTOCK, R.E., 1989. Control of Alternaria rot on sweet cherry. *Fungic. Nematicide Tests* **44**, 57.
- KALRA, J.S. & SORI, H.S., 1985. Studies on post-harvest rots of tomato fruit. Control of Alternaria fruit rot. *Indian J. Mycol. Plant Pathol.* **15**, 256-260.
- KREMER, W. & UNTERSTENHÖFER, G., 1967. Computation of results of crop protection experiments by the method of Townsend and Heuberger. *Pflanzenschutz-Nachr. Bayer* **20**, 625-628.
- LARSEN, H.J., COVEY, JR., R.P. & FISCHER, W.R., 1980. A red spot fruit blemish in apricots. *Phytopathology* **70**, 139-142.
- LASZLO, J.C., COMBRINK, J.C., EKSTEEN, G.J. & TRUTER, A.B., 1981. Effect of temperature on the emission of sulphur dioxide from gas generators for grapes. *Decid. Fruit Grower* **31**, 112-119.
- MANGIAROTTI, A.M., PICCO, A.M., CRIPPA, A. & SAVINO, E., 1987. Fungi on phylloplane of treated and not treated vineyard. *Riv. di Patol. Veget.* **23**, 27-37.
- McCOLLOCH, L.P. & WORTHINGTON, J.T., 1952. Low temperature as a factor in the susceptibility of mature-green tomatoes to Alternaria rot. *Phytopathology* **42**, 425-427.
- NELSON, K.E., 1983. Effects of in-package sulfur dioxide generators, package liners, and temperature on decay and desiccation of table grapes. *Am. J. Enol. Vitic.* **34**, 10-16.
- PEARSON, R.C. & HALL, D.H., 1975. Factors affecting the occurrence and severity of blackmold of ripe tomato fruit caused by *Alternaria alternata*. *Phytopathology* **65**, 1352-1359.
- PETRINI, O., 1986. Taxonomy of endophytic fungi of aerial plant tissues. In: FOKKEMA, N.J. & VAN DEN HEUVEL, J. (eds) Microbiology of the phyllosphere. Cambridge University Press, Cambridge. pp. 175-187.
- PROFIC-ALWASIAK, H. & SZCZYGIEL, A., 1980. Occurrence and control of Alternaria black spot of gooseberry in Poland. *Fruit Sci. Rep.* **7**, 35-43.
- PRUSKY, D., BEN-ARIE, R. & GUELFAT-REICH, S., 1981. Etiology and histology of Alternaria rot of persimmon fruits. *Phytopathology* **71**, 1124-1128.
- PRUSKY, D., FUCHS, Y. & YANKO, U., 1983. Assessment of latent infections as a basis for control of postharvest disease of mango. *Plant Dis.* **67**, 816-818.
- SIEBER, T.N., SIEBER-CANAVESI F. & DORWORTH, C.F., 1990. Simultaneous stimulation of endophytic *Cryptodiaporthe hystrix* and inhibition of *Acer macrophyllum* callus in dual culture. *Mycologia* **82**, 569-575.
- SIMMONS, E.G., 1967. Typification of Alternaria, Stemphylium, and Ulocladium. *Mycologia* **59**, 67-92.
- SMILANICK, J.L., HARTSELL, P.L., HENSON, D., FOUSE, D.C., ASSEMI, M. & HARRIS, C.M., 1990. Inhibitory activity of sulfur dioxide on the germination of spores of Botrytis cinerea. *Phytopathology* **80**, 217-220.
- SWART, A.E. & HOLZ, G., 1991. Alternaria alternata rot of cold-stored table grapes in the Cape Province of South Africa. *Phytophylactica* **23**, 217-222.
- UNTERSTENHÖFER, G., 1963. The basic principles of crop protection field trails. *Pflanzenschutz-Nachr. Bayer* **16**, 81-164.
- VERHOEFF, K., 1974. Latent infections by fungi. *Annu. Rev. Phytopathol.* **12**, 99-110.
- WALL, M.M. & BILES, C.L., 1993. Alternaria fruit rot of ripening chile peppers. *Phytopathology* **83**, 324-328.
- WILLIAMS, E., 1987. Effect of fungicide seed treatments on wheat seedling emergence. *Fungic. Nematicide Tests* **42**, 276.