Endophytic fungi associated with core rot of apples in South Africa, with specific reference to *Alternaria* species

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Apple fruits were sampled at five stages of development for endophytic colonisation by fungi associated with core rot, a major post-harvest disease. In addition, isolations were made from diseased core tissues of apples after 8 months of cold storage. The cultivars Top Red (susceptible to core rot) and Granny Smith (resistant to core rot) were sampled in orchards during the 1995/6 growing season. Of the 40 different fungal taxa encountered, 19 had a relative importance (RI) value of more than 10%. In general, more fungal isolates were obtained from Top Red than from Granny Smith apples, but no tissue specificity was detected. As found in previous studies, the *Alternaria* complex was the most dominant, representing 57% of the total number of 1602 isolates. On the basis of sporulation patterns and spore morphology, this complex could be divided into two different groups in the *Alternaria alternata*, two in the *Alternaria infectoria*, and one in the *Alternaria tenuissima* complexes. A further group was identified which may represent an additional *Alternaria* species. This study has further shown that the *Alternaria* spp. are present as endophytes already at the bud development stage, which has serious implications for any programme using fungicides for disease control.

Keywords: *Alternaria alternata*, apples, core rot, fungal endophytes

Endophytic fungi have been isolated from tissue of numerous plant species since the latter half of the 1970s (Hata & Futai, 1996). In several different studies, endophytes have been shown to protect their hosts against beetles and insects (Webber & Gibbs, 1984; Petrini & al., 1989), or cattle (Carroll, 1988; Scott & Schardl, 1993), to stimulate seed germination (Luginbühl & Müller, 1980), increase growth (Leuchtmann & Clay, 1988), and cause disease (Johnson & al., 1992; Smith & al., 1996). Species of *Alternaria* are commonly present within the endophytic mycoflora of a wide range of plants. They have been well documented in several crops including barley (Riesen & Close, 1987), wheat (Crous & al., 1995), mango (Johnson & al., 1992; Prusky, 1993), cotton (Bashan, 1994), tomato (Blancard & al.,

1984), cherry fruit (Dugan & Roberts, 1994) and sweet pepper (Halfon-Meiri & Rylski, 1983). Although they are sometimes harmless endophytes, *Alternaria* spp. have been associated with several economically important plant diseases (Rotem, 1994), and have also been cited as the main cause of dry core rot of apples (Combrink & Ginsburg, 1973; Ellis & Barrat, 1983; Combrink & al., 1985a, 1985b; De Kock & al., 1991).

Wet and dry core rot of apples (*Malus domestica* L.) are important post-harvest diseases in South Africa (Combrink & Ginsburg, 1973) and have been associated with crop losses between 5–8%. Dry core rot is associated with *Alternaria* spp., and has distinct symptoms from wet core rot, which is mainly caused by *Penicillium* spp. (Fugler, 1990). Mouldy core refers to the restricted growth of the pathogen inside the fruit's locules, and this can develop into dry core rot if the pathogen penetrates the flesh of the fruit (Spotts, 1988). As well as being a post-harvest disease problem, dry core rot has been associated with losses in the orchard before harvest, because of early fruit fall (Taylor, 1955; Combrink & Ginsburg, 1973). Several facultative parasitic fungi have been isolated from diseased apples, of which *Alternaria alternata* (Fr.: Fr.) Kiessl. has proven to be the most dominant (Combrink & al., 1985b). Facultative parasites growing saprobically in the seed cavity, from where they eventually penetrate surrounding mesocarp tissue, are the main cause of core rot (Harrison, 1935; Brien, 1937; Carpenter, 1942). Floral parts tend to be rapidly colonized by fungi after full bloom. These fungi subsequently enter the core region through the open sinus (Combrink & al., 1985a), where they are protected against fungicides (Ellis & Barrat, 1983).

The most and least affected cultivars in South Africa are respectively the Red Delicious Types (including Top Red) and Granny Smith. One probable reason for their differing susceptibility is that Top Red apples, unlike Granny Smith, have an open calyx tube. Top Red apples also have a low fumaric and malic acid content. Combrink (1983) suggested that these factors could predispose Top Red apples to infection. Core rot symptoms have been detected as early as 3-6 weeks before harvesting, suggesting early infection. In view of the prominent role that *Alternaria* spp. play in core rot (Combrink & al., 1985a, 1985b; De Kock & al., 1991), as well as their well documented endophytic growth habit in fruit (Prusky & al., 1993; Dugan & Roberts, 1994), the aim of this study was to determine which fungi, and specifically which *Alternaria* spp. occurred endophytically in apparently healthy apple fruit, at what stage infection occurred, and if the same fungi were also present in apple cultivars not susceptible to core rot.

Material and methods

Ten trees were randomly chosen from a Top Red, and another ten from a Granny Smith orchard. Four samples were collected per tree at bud stage, full bloom, fruit set, fruit 4 cm in diam., and just before harvest. The samples for each stage were respectively buds, blossoms, young fruit just after fruit set, young fruit one month later and mature fruit one day before picking. Samples were dissected using sterilised scalpels and tweezers under the hood of a laminar flow cabinet. Buds were divided into scales, bud primordia and woody stem (1 cm in length). Each flower was separated into petals, sepals, stamens, pistils, pedicel and woody stem (1 cm in length). Fruit just after fruit set were divided into sepals, stamens, pistils and pedicel with one-third length (3 mm) of the young fruit still attached. Young fruit (4 cm diam.), were separated into stamens, pistils, sepals, pedicel and its attached piece of fruit (5 mm in length). All fleshy parts of mature fruit were discarded, leaving the pedicel, seed cavity, calyx tube region, and the part of core between the pedicel and seed cavity. All dissected plant parts were surface sterilised to eliminate epiphytic propagules and mycelia by the following immersion sequence: 30 sec in 70% ethanol, followed by 2 min in 1% NaOCl, and 15 sec in 70% ethanol. Surface-sterilised segments were then transferred aseptically to marked positions in 90 mm Petri dishes containing potato dextrose agar (PDA) (Biolab). In addition, 46 Top Red and 50 Granny Smith apples, stored for 8 months (–0.5 C, controlled atmosphere), were surface sterilized as described above, and split longitudinally through the core. Tissue showing signs of core rot or mouldy core was dissected, and plated onto 90 mm Petri dishes containing PDA. Dishes were incubated on the laboratory bench at 25 C, and emerging mycelia hyphal-tipped and transferred to PDA slants incubated at 25 C.

For identification, fungal isolates were transferred to Petri dishes containing potato carrot agar (PCA) (Simmons & Roberts, 1993), and were incubated for 7–10 days at 22 C under a 10/14 h cool-white fluorescent light/dark cycle. Isolates of *Alternaria* spp. were categorised into six different groups according to their sporulation patterns at 50× magnification, and conidium morphology (Simmons & Roberts, 1993). Isolates of *Penicillium* spp. were transferred to Czapek agar (Samson & Van Reenen-Hoekstra, 1988), and incubated on the laboratory bench.

For the statistical evaluation, the colonization frequency by a fungal species was defined as the total number of pieces of a given tissue colonized by a given taxon. Two matrices were used for ordination of the endophytes assemblages: the complete matrix, containing all identified taxa, as well as a reduced matrix of the raw

data of the colonization frequencies that contained only those taxa with a relative importance (dominance) index of at least 10% (Ludwig & Reynolds, 1988). These matrices were analyzed by simple correspondence analysis using the package SimCA 2.1 (Greenacre, 1990). The fungal codes used in the graphical display are given in Tab. 1. In addition, confirmatory statistical analysis was carried out when appropriate on selected frequency data using Chi-square tests, at a significance level of 0.05.

Results and discussion

Of the 40 different taxa isolated in this study, including two distinctive sterile mycelia, 19 were present at RI values of more than 10% (Tab. 1), and only 11 at standardised RI values higher than 25%. The results of the correspondence analysis carried out on the complete matrix showed a homogeneous distribution of the fungi in the different tissue types studied (data not shown). The percentage of inertia explained by the first four factors, however, was only approx. 50%, which indicated a rather poor representation of the data by the model. We therefore carried out a correspondence analysis using only those taxa with a RI of at least 5% (Fig. 1). The total inertia explained by the first four components is 61%. This shows still only a moderate goodness of fit of the data to the model, but the analysis indicates quite clearly that *Alternaria* spp. are rather evenly distributed in most tissue types, as demonstrated by their position close to the origin (0, 0) of the display, in almost exact correspondence to the position of most samples. Stamina and sepals of Top Red fruit sets, as well as petals of Top Red full bloom stage are colonised by a mycoflora composed mainly of *Botrytis cinerea*, *Cladosporium* spp., *Phoma* sp., *Pleospora herbarum*, and a white sterile mycelium. *Acremonium* sp., on the other hand, appears to be a preferential coloniser of young stages of Granny Smith, although it is also detected in young Top Red tissues. Overall, however, both types of cultivars have a similar mycoflora and no clear gradient can be seen that allows one to describe any age-related succession. In both cultivars, *Alternaria alternata* (Fr.: Fr.) Keissl. group 1 was the most prevalent taxon, followed by *Alternaria infectoria* E. G. Simmons group 1 and *Epicoccum* spp. These findings also correlate with that reported by Combrink & al. (1985a).

In the present study, the developing buds were the earliest developmental stage sampled, but even these were already colonised throughout by fungi. In general, young Top Red tissues tended to be more heavily colonised than young Granny Smith samples, but the differences were statistically significant ($P < 0.05$) only for the bud samples (Fig. 2). Sixty percent of Top Red bud primordia were colo-

Tab. 1. – Most frequent endophytic fungi isolated from Top Red and Granny Smith buds, blossoms and fruit at five different stages of fruit development. The raw frequencies are given. The figures are the total number of isolations from a given plant part; e.g. 5/2 indicates 5 isolates of *Acremonium* sp. were isolated from bud scales of Top Red apples and 2 isolates from Granny Smith bud scales. The code indicates the symbols used in the display of the statistical analysis. Only those fungi with a relative importance value of at least 10% have been considered[a].

		Bud stage			Full bloom						Fruit set				Fruit 4cm diam.					Mature fruit			
	Code[b]	SC	BP	WS	PT	SP	ST	PS	PD	WS	SP	ST	PS	PY	ST	PS	SP	PD	SE	PD	SA	CT	CR
Acremonium sp.	AC	5/2	1/4	1/3	5/10	2/3	3/10	3/7	1/1	6/7	0/1	1/0	0/1	0/0	4/8	0/0	5/2	3/2	0/0	0/0	0/0	0/0	0/0
Alternaria alternata gr 1	A1	22/1	5/1	17/14	6/12	7/3	3/1	8/2	3/4	16/6	5/4	5/5	15/13	0/0	19/18	1/1	13/16	15/17	1/5	20/10	14/7	19/14	15/10
A. alternata gr 2	A2	6/4	0/0	5/1	2/2	1/1	0/0	0/0	1/0	2/0	0/0	2/0	1/3	0/0	1/5	0/0	3/2	4/3	0/0	4/9	8/3	4/8	4/3
A. tenuissima gr	TE	3/2	1/0	4/5	0/0	0/0	1/0	0/0	1/0	5/0	0/1	1/1	5/2	0/0	1/8	1/0	1/7	6/6	0/2	8/4	3/1	3/3	3/1
A. infectoria gr 1	I1	18/12	7/2	24/11	2/3	3/3	2/1	2/1	1/1	20/5	0/2	0/1	8/ 13	0/0	14/16	1/3	13/13	12/15	1/3	21/5	13/8	16/17	10/8
Botrytis cinerea	BR	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	1/0	6/8	0/0	5/3	0/0	1/2	3/2	0/1	0/0	1/0	6/0	0/0
Chaetomium spp.	CH	2/0	6/4	2/4	9/3	3/2	1/1	0/0	2/1	2/3	1/1	1/1	2/1	0/0	1/1	0/2	0/2	0/0	1/1	0/0	1/0	6/0	0/0
Cladosporium spp.	CS	0/0	0/0	0/1	0/0	0/1	1/0	2/0	0/0	0/0	2/0	4/2	4/5	0/0	0/5	0/0	1/1	0/0	0/0	1/1	0/2	0/0	0/0
Coniothyrium sp.	CO	1/1	0/0	1/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/2	1/2	0/0
Epicoccum spp.	EP	16/6	3/1	5/1	2/0	0/0	0/0	2/1	0/0	1/0	0/0	4/0	10/3	0/0	19/9	1/0	13/9	16/4	5/0	12/9	5/0	7/1	7/13
Fusarium spp.	FU	1/0	2/0	0/0	2/1	2/1	1/0	2/0	0/0	1/1	1/0	1/0	0/0	0/0	1/0	0/0	2/0	2/0	0/0	0/0	4/0	1/0	0/0
Nigrospora sp.	NG	1/0	2/2	1/0	1/2	0/1	1/2	0/3	0/0	2/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	4/10	1/2	0/3	1/9
Pestalotiopsis sp.	PS	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/1	0/0	0/1	0/3
Phoma sp.	PH	0/1	2/0	1/0	0/1	1/0	1/0	2/0	0/0	1/0	1/2	2/0	1/1	0/0	2/1	0/0	0/0	1/2	0/1	0/0	1/0	0/0	0/0
Phomopsis sp.	PM	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/3	0/4	0/1
Pleospora herbarum	PL	0/0	0/0	0/0	9/2	0/0	1/0	0/0	0/0	0/0	4/1	2/0	3/1	0/0	1/2	0/0	1/2	0/3	0/2	0/0	1/0	1/1	0/0
Sporomiella sp.	SM	0/1	0/1	1/1	0/0	0/1	1/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Thielavia sp.	TH	0/1	0/1	0/1	1/1	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
White sterile	WS	0/0	1/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	2/0	2/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/2	0/0	1/0

[a] Isolates with a standard relative importance of less than 10% include: *Alternaria infectoria* group 2, *Alternaria* sp., *Bipolaris* spp., *Botryosphaeria obtusa*, Brown sterile mycelium, *Costantinella* sp., *Diplodia mutila*, *Gliocladium* sp., *Idriella* sp., *Nodulisporium* sp., *Pestalotia* sp., *Phialophora* sp., *Pithomyces* sp., *Ramularia* sp., *Sordaria* sp., *Sporothrix* sp., *Stachybotrys chartarum*, *Thielavia* sp., *Trichothecium roseum*, unidentified coelomycete, unidentified hyphomycete.

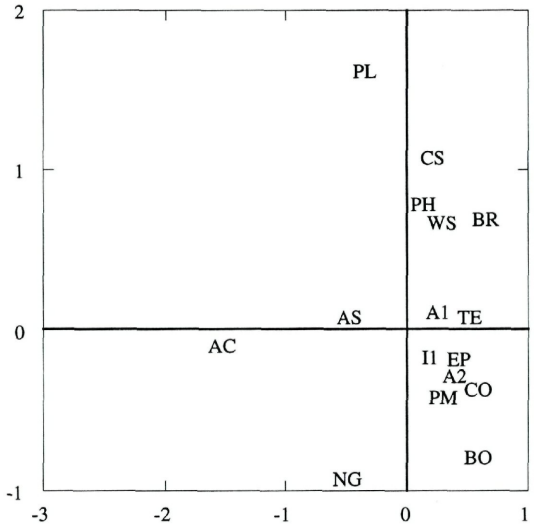
[b] Codes for plant parts: SC-scales, BP-bud primordia, WS-woody stem, PT-petals, SP-sepals, ST-stamens, PS-pistils, PD-pedicel, PY-pedicel and attached piece of fruit (3 mm in length), SE-piece of fruit (5 mm in length) attached to pedicel, SA-seed cavity, CT-calyx tube region, CR-part of core between pedicel and seed cavity.

nised, as opposed to only 22% of Granny Smith bud primordia. Top Red bud primordia also contained almost twice as many (30) endophyte isolates than bud primordia from Granny Smith (16) (Tab. 1). Previous studies have suggested that infection may occur 3-6 wk. before harvesting (Taylor, 1955; Raina & al., 1971), or during and shortly after full bloom (Ellis & Barrat, 1983; Combrink & al., 1985a), but the results of the present study, which show these fungi to occur endophytically, indicate that buds are infected much earlier than previously expected. This finding, however, should be further investigated using scanning electron microscopy, as Andrews and Kenerley (1980) have shown that surface sterilisation is not an adequate method to discriminate between external and internal bud microflora. In fact, the hairy nature of apple buds precludes effective wetting even where surfactants are used (Andrews & Kenerley, 1980) and overlapping bud scales may entrap fluid. In our procedures, however, external scales and bud primordia were separated before surface sterilisation, thus reducing the likelihood of survival of epiphytic mycelia and propagules.

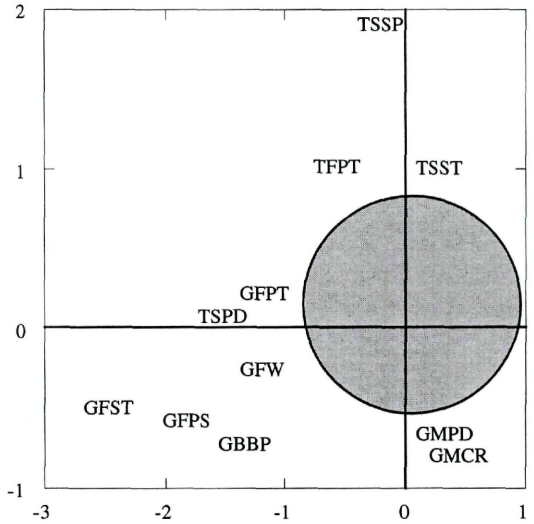
As fruit developed subsequently to the bud stage, the stamens, pistils and later the calyx tubes and seed cavities were also rapidly colonised (Figs. 3, 4). At full bloom the most prevalent taxon on stamina and pistils was an *Acremonium* sp. followed by *Alternaria alternata* group 1. This was also the stage at which the lowest number of *Epicoccum* isolates was recorded. In general, *Alternaria alternata* group 1 and *Alternaria infectoria* group 1 had the highest occurrence frequency of all taxa isolated (Tab. 1). The number of isolates slowly increased towards fruit set, which could possibly be due to the increase in calyx tube diameter after full bloom, which coincided with style separation. The number of isolates was higher still in stamina and calyx tubes of young fruit of 4 cm in diameter and of mature fruits, but no statistically significant differences were detected in the frequency of colonisation of these two tissues (Figs. 3A, 4A). Furthermore, as floral parts of mature fruit consist of dead tissue, saprophytic colonisation of these tissues could also account for some increase in the amount of isolates obtained.

Significant differences ($P < 0.05$) were seen between the infection frequencies of seed cavities for the two cultivars (Figs. 3B, 4B). The same patterns could be seen for the infection frequencies by *Alter-*

Fig. 1. – Results of the ordination by simple correspondence analysis. – A. Principal co-ordinate map of fungal taxa. – B. Corresponding map for plant pieces. – Percentage of inertia explained by the first four components: 61%. Only those taxa with a relative importance index of at least 5% have been used for the ordination analysis. Fungal codes are explained in Tab. 1. T, Top Red, G, Granny Smith. Other abbreviations as in Figs 2–3. The dashed circle in Fig. 1B covers the region in the map in which all other tissue pieces are situated.



A



B

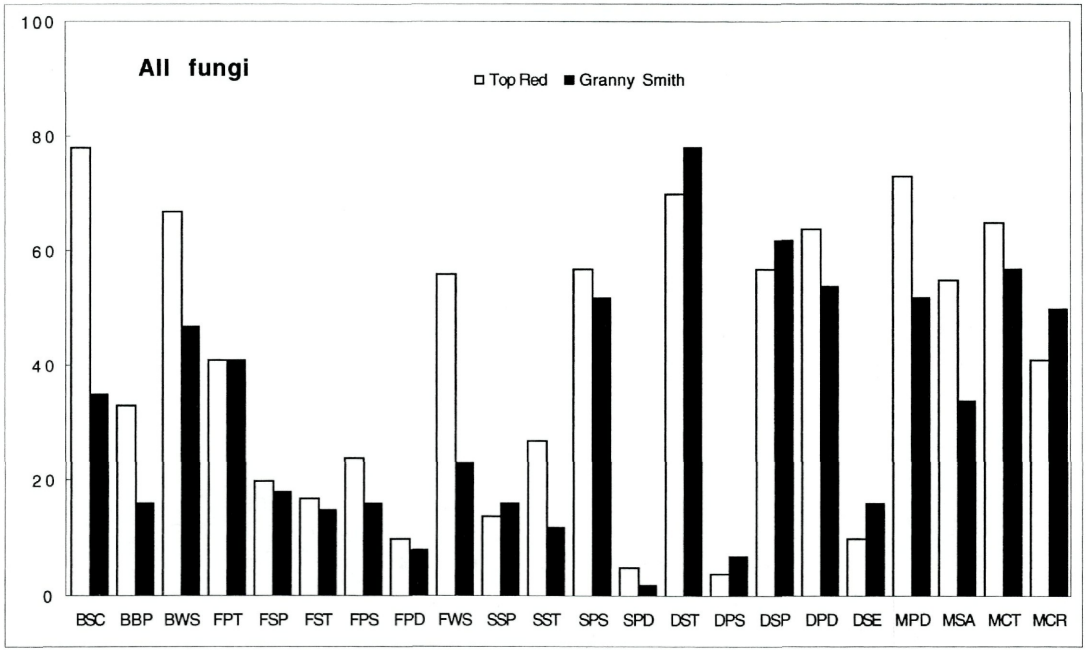
naria spp. (Fig. 4). The consistently and statistically higher incidence of infection in seed cavities of Top Red could contribute to the higher core rot incidence in Top Red than in Granny Smith apples. No core rot symptoms extending into the mesocarp were, however, detected in any of the apples sampled. This is not totally unexpected, as the disease frequently only develops at a later stage in the packhouses.

Isolations made from stored Top Red apples showed that 23 of the 46 samples tested had internal symptoms of either mouldy core or core rot. All the stored Granny Smith apples were healthy. Six of the Top Red apples with wet rot also had external brown lesions. The two *Penicillium* spp. isolated from these lesions were *P. expansum* Link and *P. funiculosum* Thom (Samson & Van Reenen-Hoekstra, 1988). Results show that only 6.5% of the Top Red apples had symptoms of core rot. This figure confirms the results by Fugler (1990). Thus, even though the seed cavities of all the mature Top Red apples were colonized by *Alternaria* spp. before storage (Tab. 1), only a very small percentage actually developed core rot. This finding suggests that other factors may also play a role in disease development. Pathogenicity tests now need to be conducted to correlate disease symptoms with the *Alternaria* spp. isolated from apple tissues.

Core rot of red apple cultivars is presently regarded as one of the most serious diseases of harvested apples in South Africa, resulting in huge annual losses of income, which extrapolates further due to a reduced consumer confidence in South African fruit produce. Since the exact time of infection is still unclear, the application of cost effective control measures is often very difficult (Fugler, 1990).

Most apple cultivars susceptible to core rot have an open calyx tube (Spotts, 1988), and Pierson & al. (1971) speculated that the pathogen enters the seed cavity via this route. However, Combrink & al. (1985a) found that at harvest more than 50% of all fruit and seed cavities of apples with either an open (Starking) or a closed calyx tube (Golden Delicious and Granny Smith) were colonised by fungi, suggesting that other factors were also involved with disease development. Combrink (1983) speculated that the low fumaric and malic acid content of Top Red apples could predispose them to infection. Ellis and Barrat (1983) stated that disease development could possibly be accelerated by frost, while Miller (1959) found that a ruptur-

Fig. 2. – Incidence of fungal taxa recovered from all tissues examined. Codes of plant parts: SC, scales; BP, bud primordia; WS, woody stem; PT, petals; SP, sepals; ST, stamens; PS, pistils; PD, pedicel; PY, pedicel and attached piece of fruit (3 mm in length); SE, piece of fruit (5 mm in length) attached to pedicel; SA, seed cavity; CT, calyx tube region; CR, part of core between pedicel and seed cavity. The first letter indicates the age of the tissue: B, Bud stage; F, Full bloom; S, Fruit set; D, Fruit 4 cm diameter; M, Mature fruit.



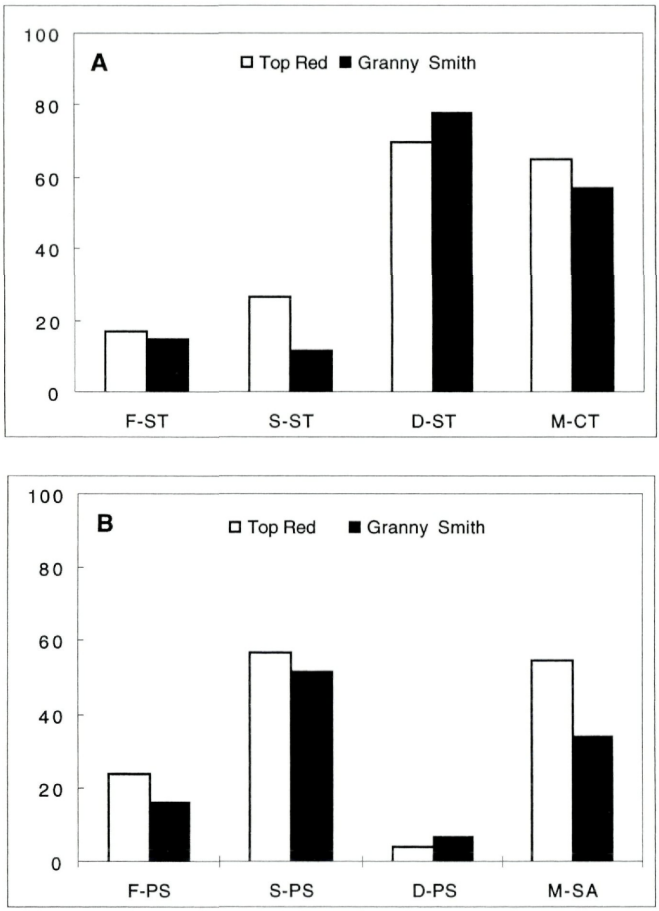
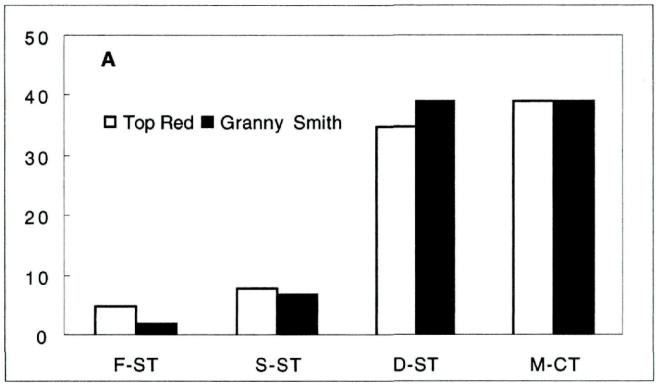


Fig. 3. – Incidence of fungal taxa recovered from stamens and calyx tubes (A) and from pistils and seed cavities (B) of Top Red and Granny Smith flowers and fruits collected at various stages of fruit development. – Codes of plant parts: SC, scales; BP, bud primordia; WS, woody stem; PT, petals; SP, sepals; ST, stamens; PS, pistils; PD, pedicel; PY, pedicel and attached piece of fruit (3 mm in length); SE, piece of fruit (5 mm in length) attached to pedicel; SA, seed cavity; CT, calyx tube region; CR, part of core between pedicel and seed cavity. The first letter indicates the age of the tissue: F, Full bloom; S, Fruit set; D, Fruit 4 cm diameter; M, Mature fruit.



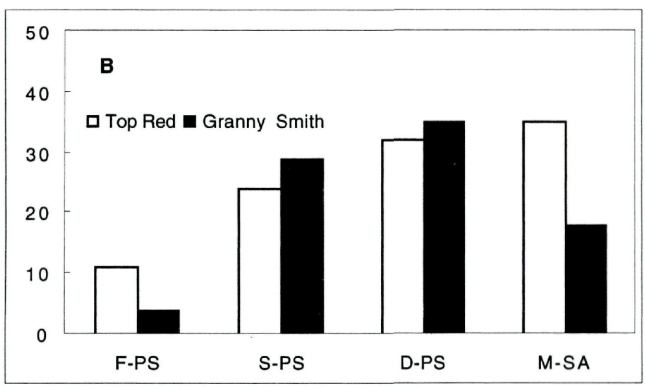


Fig. 4. – Incidence of *Alternaria* spp. recovered from stamens and calyx tubes (A) and from pistils and seed cavities (B) of Top Red and Granny Smith flowers and fruits collected at various stages of fruit development. Codes of plant parts: SC, scales; BP, bud primordia; WS, woody stem; PT, petals; SP, sepals; ST, stamens; PS, pistils; PD, pedicel; PY, pedicel and attached piece of fruit (3 mm in length); SE, piece of fruit (5 mm in length) attached to pedicel; SA, seed cavity; CT, calyx tube region; CR, part of core between pedicel and seed cavity. The first letter indicates the age of the tissue: F, Full bloom; S, Fruit set; D, Fruit 4 cm diameter; M, Mature fruit.

ing of the calyx tissue due to abnormally fast growth following heavy rains after a dry period also played a role. This observation was further substantiated by Combrink & al. (1985a), who found growth cracks appearing in the cavities of Red Delicious types 2 wk. after full bloom, and a sporulating *Alternaria* sp. in the calyx tube 16 wk. after full bloom. Conidia of this fungus may accumulate on plant surfaces during dry weather, where they are able to survive ultraviolet radiation due to their pigmented, multi-celled conidia, thus accounting for elevated disease levels after dry periods. As soon as free moisture appears, these conidia may germinate, thus causing infection (Rotem & Aust, 1991; Rotem, 1994).

Isolates of *Alternaria* spp. could be accommodated within six different groups according to their sporulation patterns and conidium morphologies (Simmons & Roberts, 1993). Two groups were classified as *Alternaria alternata*, two as *Alternaria infectoria,* one as *Alternaria tenuissima* and one as an *Alternaria* sp. *Alternaria alternata* group 1 and *Alternaria infectoria* group 1 were the dominant *Alternaria* taxa. The large number of *Alternaria* spp. isolated throughout the season does not, however, imply that they are also the dominant fungi inducing core rot, as other fungi may also play a role in disease development. Further inoculations and pathological studies are now required to determine the relative importance of these fungi.

Since the last decade, several new studies have emphasized that *Alternaria* spp. have a role as toxigenic fungi and not simply as plant pathogens. Alternariol (AOH) and alternariol monomethyl ether (AME) are two toxins readily produced by *Alternaria alternata*, even in the absence of mycelium (Vinãs & al., 1992; Robiglio & Lopez, 1995). These metabolites are toxic to bacteria (Ozcelik & Ozcelik, 1990), animals (Griffin & Chu, 1983) and human cell cultures (Harvan & Pero, 1976). Both AOH and AME have been shown to be produced by *Alternaria alternata* strains isolated from Red Delicious apples with mouldy core symptoms (Ozcelik & al., 1990; Robiglio & Lopez, 1995). A synergistic toxicity has been found to exist between AOH and AME (Pero & al., 1973), which could play a very important role in human esophageal cancer (Liu & al., 1991, 1992). Another endophyte isolated from apples in this study, *Stachybotrys chartarum* (Ehrenb. ex Link) S. Hughes, has also been linked to mycotoxicosis of various animals, including humans (Marasas & Nelson, 1987).

Two *Alternaria* isolates (BAFC 525, 2174), associated with core rot of apple in Argentina (Robiglio & Lopez, 1995) were studied by us, and subsequently identified as representative of the *Alternaria tenuissima* group. The only *Alternaria* group isolated from dry rot lesions on stored apples in the present study also belongs to this group.

This study has shown that several fungi, especially potentially toxigenic *Alternaria* spp., are already well established in apple fruit at the bud stage of development. This suggests that the fungi associated with core rot are in fact dormant inside the tissue, waiting for suitable conditions to cause disease. Given the early colonisation of apple tissue, early fungicide applications would thus be worth considering in the management of orchards prone to core rot disease.

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