THE ASSOCIATION OF *TARSONEMUS* MITES (ACARI: HETEROSTIGMATA) WITH DIFFERENT APPLE DEVELOPMENTAL STAGES AND APPLE CORE ROT DISEASES

Lené Van der Walt[1], Robert A. Spotts[2], Eddie A. Ueckermann[3,4], Francois J. Smit[1], Tamaryn Jensen[1] and Adéle McLeod[1]

1. Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa (e-mails: LeneV@nda.agric.za, Francois.Smit@za.samiller.com, tammy@sun.ac.za, and adelem@sun.ac.za); 2. Mid-Columbia Agricultural Research and Extension Centre, Oregon State University, Hood River, OR 97031, USA (e-mail: Robert.spotts@oregonstate.edu); 3. Agricultural Research Council, Plant Protection Research Institute, Private Bag X134, Queenswood, Pretoria 0121, South Africa (e-mail: UeckermannE@arc.agric.za); 4. School of Environmental Sciences and Development, North-West University, Potchefstroom Campus 2520, South Africa

(Received 29 July 2010; accepted 2 November 2010)

ABSTRACT – Information on the role of mites in the genus *Tarsonemus* Canestrini and Fanzago, 1876 in the epidemiology of apple core rots (wet and dry) is limited. The aims of this study were to (1) assess the effect of different apple developmental stages (buds, blossoms, 4-cm diameter fruit, mature fruit and mummies) on the relative abundance of *Tasonemus* mites, (2) determine if there is a tendency of *Tarsonemus* mites to be associated with wet core rot (WCR) and dry core rot (DCR) apples, and (3) evaluate the suitability of three core-rot-associated fungal genera as food sources for the mites. Investigations into four orchards, two core-rot-susceptible (Red Delicious) and two-core-rot resistant (Granny Smith), revealed that *Tarsonemus* mites were the dominant mite genus in all the apple developmental stages in all orchards. The *Tarsonemus* mites had the highest incidence in mature fruits and mummies in all the orchards. In the cores of healthy and DCR Red Delicious fruits, *Tarsonemus* mites had a high occurrence of 56% and 84%, respectively. In these fruits, a significant association was found between DCR and the presence of mites in the core. In contrast, in Granny Smith fruits, mites were restricted to the calyx tubes, and only a calyx tube decay symptom was identified. The *Tarsonemus* mites were fungivorous and reproduced on cultures of a *Cladosporium* sp. Cultures of *Alternaria* sp. and *Penicillium* sp. were unsuitable for mite reproduction, even though the mites did ingest a red fluorescently labeled *Alternaria* sp. culture. The survival and reproduction of mites on fungal cultures were better at 30˚C than at 25˚C.

Key words – Tarsonemidae, apple core rot, ecology, vectors.

INTRODUCTION

Tarsonemus Canestrini and Fanzago, 1876 mites are common in nature and have been collected from many plant species, fungi, leaf litter, soil, and stored food products (Lindquist, 1986; Zhang, 2003). The genus *Tarsonemus* belongs to the family Tarsonemidae, subfamily Tarsoneminae, and contains more than 200 described species (Lindquist, 1986). The majority of species within the genus are considered to be fungivorous (McDaniel, 1979; Lindquist, 1986). However, *Tarsonemus confusus* Ewing, 1939 occasionally occurs as a minor pest in greenhouses where they can cause damage to plants (Zhang, 2003).

The fungivorous nature of *Tarsonemus* mites creates the potential for the vectoring of fungi, and they can therefore play a role in fungal dispersal and ecology. Studies on *Tarsonemus* fungal dispersal have

focused on the vectoring of Ophiostomatoid fungi in the northern hemisphere (Lombardero *et al*., 2003; Hofstetter *et al*., 2006). In these regions, *Tarsonemus* mites may be important in the dispersal of plant pathogenic *Ophiostoma* spores among various coniferous trees (Dowding, 1969; Moser *et al*., 1995; Klepzig *et al*., 2001). The best example is the vectoring of the blue-stain fungus, *Ophiostoma minus* (Hegdc.) J. Hunt, 1956 by three *Tarsonemus* spp. that are phoretic on the Southern pine beetle, *Dendroctonus frontalis* Zimmerman (Moser, 1976). In South Africa, non-pathogenic *Ophiostoma* sp. in *Protea* infructescences may also be vectored by *Tarsonemus* mites that are carried on beetles (Roets *et al*., 2007, 2009).

Tarsonemus mites may also play a role in core rot diseases of apples. A preliminary study in California, USA, provided some support for the role of *Tarsonemus* mites in the epidemiology of core rot diseases, specifically dry core rot (DCR) caused by *Coniothyrium* (Michailides *et al*., 1994). The study found a high incidence of *T. confusus* in the cores of apples with DCR, but not in healthy apples. It was also shown in preliminary experiments that inoculation of apples with *Coniothyrium* and mites resulted in a higher incidence of DCR than when apples were only inoculated with *Coniothyrium*. The authors hypothesized that *Tarsonemus* mites may carry *Coniothyrium* sp. spores into the apple core through the open calyx tube. The mites may also cause small wounds in the core that facilitate pathogen entry and disease development (Michailides *et al*., 1994).

Apple core rots are economically important in many countries, including the United States, South Africa, Australia, New Zealand, Canada, Israel, United Kingdom and the Netherlands (Spotts, 1990; Niem *et al*., 2007). The disease is often severe in Red Delicious, Golden Delicious, Gravenstein and Idared cultivars, which all have fruits with a high incidence of open calyx tubes (Spotts, 1990). Core rots are rare in cultivars such as Granny Smith that seldom have fruits with an open calyx tube (Combrink, 1983). Based on the symptoms and causal agents, apple core rots can be divided into DCR and wet core rot (WCR). Both WCR and DCR consist of a rot that spreads from the core region into the fleshy tissue surrounding the seed cavity (Spotts *et al*., 1988). Before harvest, core rots can also reduce yields because they cause apples to drop prematurely in the orchard (Combrink and Ginsburg, 1973; Michailides *et al*., 1994).

The specific fungal pathogens that cause WCR and DCR diseases include several fungal genera. The reported causal agents of DCR include *Alternaria* spp., *Cladosporium* spp., *Coniothyrium* sp., *Epicoccum* spp., *Pleospora herbarum* Pers. Rabenh, *Stemphylium* spp., and *Ulocladium* spp. (Combrink *et al*., 1985; Spotts, 1990). However, the fungi most frequently causing DCR are small-spored *Alternaria* spp. (Serdani *et al*., 2002; Niem *et al*., 2007) including *Alternaria alternata* (Fr.) Keissler, 1912, and the *Alternaria tenuissima (Kunze)* Wiltshire, 1933 species-group (Combrink and Ginsburg, 1973; Combrink *et al*., 1985; Serdani *et al*., 1998). WCR is mainly caused by *Penicillium* spp. and sometimes by *Botryosphaeria obtusa* (Schwein.) Shoemaker, 1964, *Botrytis cinerea* Pers., 1794, *Fusarium* spp., *Mucor piriformis* E. Fischer, 1892, and *Pestalotia laurocerasi* (Westend.) Steyaert, 1949 (Spotts, 1990; Van der Walt *et al*., 2010).

The epidemiology of core rot diseases is poorly understood. It is generally accepted, but not conclusively proven, that DCR pathogens enter the core region by growing through the open calyx tube of susceptible cultivars, because these pathogens colonize blossom parts early in the season (Miller, 1959; Combrink and Ginsburg, 1973; Ellis and Barrat, 1983; Spotts, 1990). However, some studies suggested that *Alternaria* is an endophyte of various apple tissues (Serdani *et al*., 1998; Teixido *et al*., 1999) and that colonization may take place very early in the season at the bud stage (Serdani *et al*., 1998). Factors that may be involved in DCR infection and symptom development include the susceptibility of seed locules to *Alternaria* colonization (Niem *et al*., 2007), and the higher pH of mesoderm tissue adjacent to the seed locules that could enhance pathogen virulence (Combrink, 1983; Niem *et al*., 2007). WCR infections mainly occur during postharvest dipping of fruits (Combrink and Ginsburg, 1973; Combrink *et al*., 1987), but infections can also take place during the growing season (Van der Walt *et al*., 2010).

In South Africa, core rots are among the most important postharvest diseases of Red Delicious strains, causing postharvest losses between 5% and 8%. Furthermore, as fruits with core rot generally do not show visible external symptoms, diseased fruit cannot be removed in packing lines. When these fruits reach the consumer it not only affects consumer confidence (Combrink and Ginsburg, 1973; Serdani *et al*., 1998; McLeod *et al*., 2008; Van der Walt *et al*., 2010) but have also resulted in the discontinuation of export of several Red Delicious strains. A reduction in losses can only be attained through an improved understanding of the epidemiology of the diseases. Therefore, the overall aim of this study was to determine whether mites may play a role in the epidemiology of the disease in South Africa. This was investigated by (1) assessing the effect of different apple developmental stages on the relative abundance of *Tarsonemus* mites; (2) determining if there is a tendency of *Tarsonemus*

mites to be associated with WCR and DCR, and (3) evaluating the suitability of the fungi *Alternaria* sp., *Penicillium* sp., and *Cladosporium* sp. as food sources for *Tarsonemus* mites.

MATERIALS AND METHODS

Assessing the effect of different apple developmental stages on the relative abundance of *Tarsonemus* mites – Four apple orchards were selected in the Ceres area of South Africa. Trees in two of the orchards (CSC1 and CSC2) were cultivar Red Delicious (strain Oregon Spur) with a history of core rot. Trees in the other two orchards (CRC1 and CRC2) were Granny Smith with no known core rot history. In each of the four orchards 25 trees were randomly selected and marked for mite sampling at four fruit developmental stages. In the 2005/2006 season, buds (September 2005) and mature fruits (April 2006) were sampled in only one of the orchards (CSC1). Developmental stages that were sampled in the 2006/2007 season in all four orchards included full bloom (October 2006), fruit at 4-cm diameter (November 2006), mature fruit just before harvest (March 2007), and mummies (undeveloped fruit from the previous season that mummified) within trees. Mummies were collected from trees at the start of and during the growing season. In each orchard, five buds, blossoms, 4-cm diameter fruits, and mature fruits were collected from each of the 25 trees.

Using a stereomicroscope at 40× magnification plant parts were inspected for mites. Buds (only orchard CSC1) were dissected, and 4-cm diameter fruits, mature fruits, and mummies were cut longitudinally, allowing for the examination of the core cavity and the calyx tube. The location of mites and their identity to family level were recorded. Mites within the family Tarsonemidae were identified to genus level. Identification of mites to the family, genus, and species (only *Tarsonemus*) levels was conducted by mounting the mites on slides, and examination under 100× magnification.

Tendency of *Tarsonemus* mites to be associated with the core and calyx of healthy and diseased mature apple fruits – *Sampling strategy* – The association of *Tarsonemus* mites with healthy and core-rot-diseased mature fruits was studied in 11 Red Delicious (Table 1) and four Granny Smith (Table 2) orchards from the 2005/2006 to 2007/2008 seasons. Red Delicious orchards were situated in three of the main apple production regions of South Africa including Ceres (orchards with CSC prefix), Ermelo (orchards with CSE prefix), and Grabouw (orchards with CSG prefix) (Table 1). The orchards with Granny Smith fruits were all situated in Ceres (orchards with CRC prefix) (Table 2). In each orchard, 40–304 fruits were sampled by randomly harvesting up to four mature fruits per tree throughout the orchard. All the fruits were inspected for the presence of core rots, whereas only subsets that represented the different orchards (Tables 1 and 2) were analyzed for the presence of mites because of the time-consuming nature of microscopically searching for mites within fruits.

Determination of core rot diseases (WCR and DCR) and mites in the core and calyx of mature fruits – Apple fruits were cut longitudinally through the core and calyx tube and inspected for mites using a stereomicroscope at 25× and 40× magnification. Identification of core rot diseases was based on specific visual symptoms in tissue surrounding the core, with WCR and DCR being identified as a wet or dry rot, respectively, extending from the core into the fleshy tissue. The causative agents of WCR and DCR in sampled fruits were identified through isolation studies from lesions, as mainly consisting of *Penicillium* spp. and *Alternaria* spp., respectively, as expected (McLeod *et al*., 2008; Van der Walt *et al*., 2010). No core rot was observed in Granny Smith fruits, but incidence of decay in the calyx tube was recorded.

Statistical analyses – Statistical analyses were conducted to determine if there was a tendency for *Tarsonemus* mites to be associated with the core and calyx of healthy and diseased mature apple fruits. Results of the presence and absence of disease symptoms and mites were reported in 2 × 2 contingency tables that were used in association analyses (Clewer and Scarisbrick, 2006). A contingency table provides a technique for investigating suspected relationships. The null hypothesis that was tested was whether two characteristics occur independent of one another, that is, the probability that an individual falls in a certain class is not affected by another class to which that individual happens to belong. When the variables are independent, it means that knowledge of one provides no information about the other variable (Clewer and Scarisbrick, 2006). Because core rots occurred at low frequencies and were considered to be rare events, Fisher's exact test, which is applicable for small sample sizes and sparse tables, was used (Clewer and Scarisbrick, 2006). For Red Delicious fruits the null hypothesis was that occurrence of WCR and DCR was independent of the presence of mites in the calyx or core. For Granny Smith fruits the null hypothesis was that the occurrence of calyx tube decay was independent of the presence of mites in the calyx tube.

Table 1. Incidence of dry and wet core rot diseases and *Tarsonemus* mites in Red Delicious (strains Oregon Spur and Top Red) mature fruits collected from orchards in different apple production regions of South Africa.

							Fruits with mites in calyx tube			Fruits with mites in core region			
Orchard	Strain	Season	Region	DCR fruits (%)[a]	WCR fruits (%)[a]	Fruits analyzed for core rots	Healthy (%)[a]	DCR (%)[a]	WCR (%)[a]	Healthy (%)[a]	DCR (%)[a]	WCR (%)[a]	Fruits analyzed for mites
CSC1	Oregon Spur	2005/2006	Ceres	1 (0.4)	4 (1.7)	238	48 (20.6)	0 (0.0)	1 (25.0)	106 (45.5)	1 (100)	1 (25.0)	238
CSC1	Oregon Spur	2006/2007	Ceres	18 (6.0)	4 (1.3)	300	15 (14.7)	0 (0.0)	0(0.0)	67 (65.7)	15 (83.3)	4 (100)	124
CSC2	Oregon Spur	2006/2007	Ceres	17 (5.7)	2 (0.7)	300	18 (17.8)	0 (0.0)	0 (0.0)	67 (66.3)	17 (100)	1 (50.0)	120
CSC3	Oregon Spur	2006/2007	Ceres	8 (2.7)	3 (1.0)	300	7 (18.0)	2 (25.0)	0 (0.0)	25 (64.1)	4 (50.0)	2 (66.7)	50
CSC4	Oregon Spur	2006/2007	Ceres	10 (3.3)	2 (0.7)	300	6 (16.7)	0 (0.0)	0 (0.0)	21 (58.3)	9 (90.0)	1 (50.0)	48
CSC5	Oregon Spur	2006/2007	Ceres	10 (3.3)	3 (1.0)	300	5 (13.9)	0 (0.0)	0 (0.0)	20 (55.6)	9 (90.0)	2 (66.7)	49
CSC6	Oregon Spur	2006/2007	Ceres	8 (2.7)	5 (1.7)	300	10 (25.0)	0 (0.0)	0 (0.0)	23 (57.5)	7 (87.5)	5 (100)	53
CSE	Top Red	2006/2007	Ermelo	17 (5.7)	1 (0.3)	300	9 (13.6)	1 (5.9)	0 (0.0)	44 (66.7)	16 (94.1)	1 (100)	84
CSG1	Top Red	2006/2007	Grabouw	8 (2.8)	1 (0.3)	290	1 (2.9)	0 (0.0)	0 (0.0)	19 (55.9)	5 (62.5)	1 (100)	43
CSG2	Top Red	2006/2007	Grabouw	17 (5.7)	0 (0.0)	300	2 (5.7)	0 (0.0)	0 (0.0)	21 (60.0)	15 (88.2)	0 (0.0)	52
CSG3	Top Red	2006/2007	Grabouw	5 (1.8)	2 (0.7)	282	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)	1 (50.0)	15
CSG4	Top Red	2006/2007	Grabouw	5 (1.7)	1 (0.3)	290	5 (35.7)	0 (0.0)	0 (0.0)	4 (28.6)	3 (60.0)	0 (0.0)	20
Total (average)				124 (3.5)	28 (0.8)	3500	129 (17.3)	3 (2.42)	1 (3.57)	417 (56.0)	104 (83.9)	19 (67.9)	896

Note: [a]The number of apples with the specific disease symptom, dry core rot (DCR) and wet core rot (WCR), or mites present is followed by the percentage of apples with this condition in parentheses.

Table 2. Incidence of calyx tube decay and *Tarsonemus* mites in the calyx tube of mature Granny Smith fruits collected from apple orchards in the Ceres production region of South Africa.

Orchard	Cultivar	Season	Fruits with calyx tube decay[a]	Total number of fruits analyzed for decay	Healthy fruits with mites[a]	Calyx tube decay fruits with mites[a]	Total number fruits analyzed for mites
CRC1	Granny Smith	2006/2007	12 (9.6)	125	66 (58.4)	7 (58.3)	125
CRC1	Granny Smith	2007/2008	5 (1.7)	294	0 (0.0)	2 (40.0)	10
CRC2	Granny Smith	2006/2007	3 (7.5)	40	19 (51.4)	2 (66.7)	40
CRC2	Granny Smith	2007/2008	9 (3.5)	254	3 (37.5)	6 (66.7)	17
CRC3	Granny Smith	2007/2008	6 (2.6)	227	1 (12.5)	1 (16.7)	14
CRC4	Granny Smith	2007/2008	1 (0.3)	304	3 (17.7)	1 (100)	18
Total (average)			36 (2.9)	1244	92 (48.9)	19 (52.8)	224

Note: [a]The number of apples with the specific condition (calyx tube decay and healthy) or mites present is followed by the percentage of apples with this condition in parentheses.

The ability of *Tarsonemus* mites to complete their life cycle on *Alternaria* sp., *Penicillium* sp., and *Cladosporium* sp. cultures – *Alternaria* sp., *Penicillium* sp., and *Cladosporium* sp. were isolated from diseased apple core tissue. The genera were chosen to specifically represent the causative genera of DCR (*Alternaria*) and WCR (*Penicillium*), as well as a genus (*Cladosoprium*) that has most frequently been isolated from non-surface-sterilized *Tarsonemus* mites in South Africa and is also frequently isolated from apple core regions (Van der Walt, 2009; Van der Walt *et al*., 2010).

Mites were obtained from the core of Oregon Spur mummies. The adult mites, which included males and females, were transferred to 7-day-old cultures of single isolates of *Alternaria* sp., red fluorescent *Alternaria* sp., *Penicillium* sp., and 5-day-old cultures of *Cladosporium* sp. growing on potato dextrose agar (PDA) containing 0.04 g streptomycin per liter (PDA+). The red fluorescent *Alternaria* isolate, obtained from a DCR lesion, was labeled with the red fluorescent protein DsRed-Express, as previously described for *Phaeomoniella chlamydospora* W. Gams, Crous, MJ Wingf. & Mugnai (McLean *et al*., 2009). Ten *Tarsonemus* mites were transferred to pairs of plates, and one plate was incubated at 25°C and the other at 30°C. Controls consisted of two PDA+ plates that were not inoculated with any fungus. The plates were inspected for eggs and nymphal stages of the mites after 7 days and again at 14 days. The experiments were conducted twice.

The association of red fluorescent *Alternaria* spores and mycelium with the mites was investigated using an epifluorescent Zeiss Axioscope microscope (Carl Zeiss, Cape Town, South Africa), equipped with an HQ:TRITC filter with excitation filter of 545 nm, emission filter of 620 nm, and beam splitter Q570lp (Chroma Technology Corp., Bellows Falls, VT, USA). The mites feeding on the fluorescent culture were also viewed using a stereomicroscope. Images were captured with a Nikon digital camera DXM1200 (Nikon, Cape Town, South Africa) and AUTOMATIC CAMERA TAMER (ACT-1) computer software (Nikon, Cape Town, South Africa).

RESULTS

Assessing the effect of different apple developmental stages on the relative abundance of *Tarsonemus* mites – In all samples where mites were identified, *Tarsonemus* mites were the predominant mite genus (Table 3; Fig. 1). A random selection of the *Tarsonemus* mites that were identified to the species level revealed the presence of at least three species. Specimens of the *Tarsonemus* spp. have been deposited to the Mite Collection of ARC-PPRI and included (1) *Tarsonemus waitei* Banks (Acy/10/257–267), (2) a putative new species with closest similarity to *Tarsonemus mixtus* Kaliszewski (Acy 10/269) and (3) a second putative new species

Table 3. Mite families in Oregon Spur and two Granny Smith apple orchards at selected developmental stages.

Orchard[a]	Developmental stage and season sampled[b]	% Incidence of mite families								
		Tarsonemidae[c]	Phytoseiidae	Tetranichidae	Tydeiidae	Bdellidae	Ascidae	Oribatidae	Dolichocybidae	Acaridae
CSC1	Buds 2006/2007	20.00	–	37.60	–	–	–	–	–	–
	Blossom 2006/2007	1.60	–	–	–	–	–	–	–	–
	4-cm diameter fruit 2006/2007	6.40	–	–	–	–	–	–	–	–
	Mature fruit calyx 2005/2006	20.85	10.21	8.94	1.28	–	–	0.43	–	0.43
	Mature fruit core 2005/2006	45.96	3.40	–	–	–	–	–	–	–
	Mature fruit calyx 2006/2007	12.00	0.80	0.8	–	–	–	–	–	–
	Mature fruit core 2006/2007	68.80	–	–	–	–	–	–	–	–
	Mummies 2005/2006	100	–	–	–	–	–	–	–	–
	Mummies 2006/2007	100	–	–	–	–	–	–	–	–
CSC2	Blossom 2006/2007	0.80	–	0.80	–	–	–	–	–	–
	4-cm diameter fruit 2006/2007	5.04	–	–	0.84	–	–	–	–	–
	Mature fruit calyx 2006/2007	15.00	3.33	3.33	0.83	–	–	–	–	–
	Mature fruit core 2006/2007	70.83	0.83	0.83	2.50	–	–	–	0.83	–
	Mummies 2006/2007	100	6.25	–	–	–	–	–	–	–

(*Continued*)

Table 3. (*Continued*).

Orchard[a]	Developmental stage and season sampled[b]	% Incidence of mite families								
		Tarsonemidae[c]	Phytoseiidae	Tetranichidae	Tydeiidae	Bdellidae	Ascidae	Oribatidae	Dolichocybidae	Acaridae
CRC1	Blossom 2006/2007	–	–	–	–	–	–	–	–	–
	4-cm diameter fruit 2005/2006	11.2	0.80	–	0.80	–	–	–	–	–
	Mature fruit calyx 2005/2006	52.72	–	–	2.94	–	–	–	–	–
	Mature fruit core 2005/2006	–	–	–	–	–	–	–	–	–
	Mature fruit calyx 2006/2007	58.87	5.65	4.03	0.81	–	–	–	–	–
	Mature fruit core 2006/2007	–	–	–	–	–	–	–	–	–
	Mummies 2006/2007	100	–	–	–	–	–	–	–	–
CRC2	Blossom 2006/2007	–	–	–	–	–	–	–	–	–
	4-cm diameter fruit 2006/2007	7.76	–	–	–	–	–	–	–	–
	Mature fruit calyx 2006/2007	34.44	2.83	2.02	0.41	–	–	–	–	–
	Mature fruit core 2006/2007	–	–	–	–	–	–	–	–	–
	Mummies	100	–	–	–	5.55	–	–	–	–

Notes: [a]The CSC1 and CSC2 orchards were Oregon Spur orchards, whereas the CRC1 and CRC2 orchards were Granny Smith orchards.
[b]Developmental stages that were sampled included buds, blossoms, 4-cm diameter fruit, mature fruits, and mummies. Each mature fruit was inspected for the presence of mites in the calyx tube as well as in the core.
[c]All mites that were found within the family Tarsonemidae belonged to the genus *Tarsonemus*.

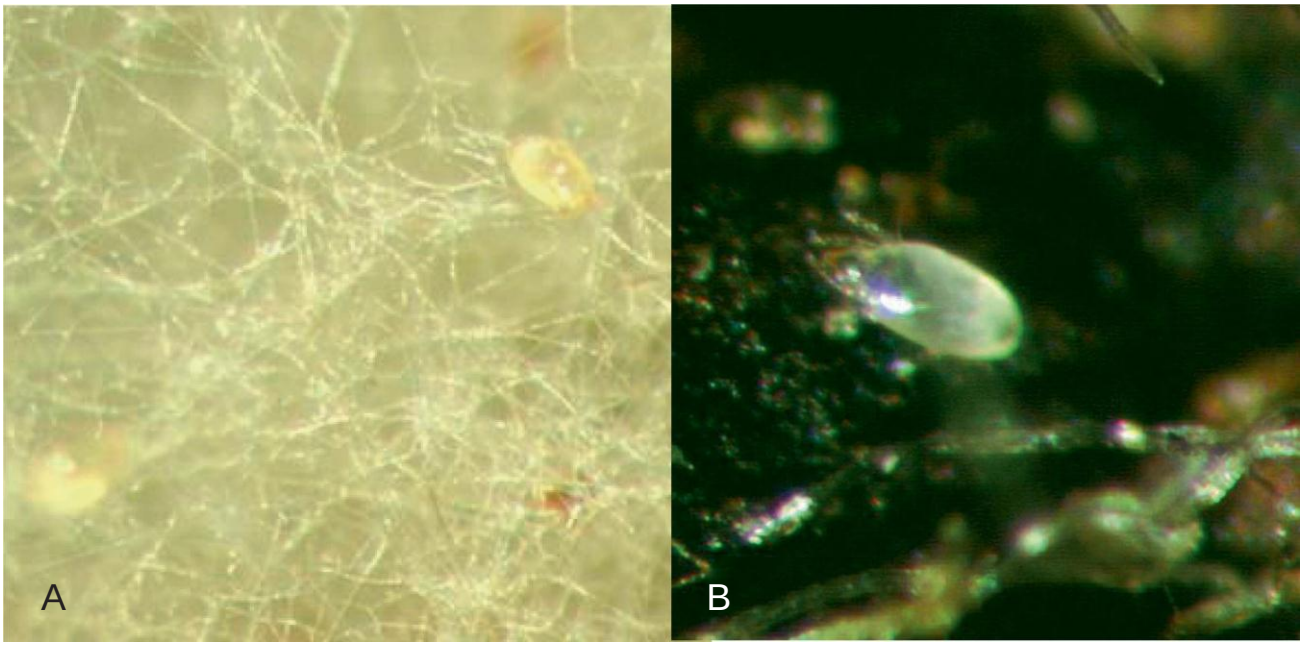

Fig. 1. *Tarsonemus* sp. – A. present in an apple core among fungal hyphae; B. in an apple mummy.

(AcY06⁄145) with closest similarity to *Tarsonemus bilobatus* Suski (personal communication, Ron Ochoa, Systematic Entomology Laboratory, U.S. Department of Agriculture). Mites from other families including Phytoseiidae, Tetranychidae, Tydeiidae, Bdellidae, Ascidae, Oribatidae, Dolichocybidae, and Acaridae were identified but were present at very low frequencies (<10%) compared with the *Tarsonemus* sp. (Table 3).

In the Oregon Spur orchards CSC1 and CSC2, *Tarsonemus* mites were present in all plant developmental stages. In orchard CSC1 where sampling was initiated in the 2005⁄2006 season, *Tarsonemus* mites were found early in the season in buds (20%) as well as blossoms, although the incidence on blossoms was <2%. As the season progressed, *Tarsonemus* mites were also found in 4-cm diameter fruit, and in the core and calyx of mature fruits (Table 3). In orchard CSC2, where blossoms were the first developmental stage sampled, *Tarsonemus* mites were present in <1% of blossoms. Mites were also found in the core and calyx region of 4-cm diameter fruits and in mature fruits from this orchard. In both Oregon Spur orchards, all the mummies contained *Tarsonemus* mites (Table 3).

In the Granny Smith orchards CRC1 and CRC2, *Tarsonemus* mites were not found in blossoms or in the core of mature fruits but were present in the calyx of 4-cm diameter fruits, as well as in the calyx tube of mature fruits. Similar to the Oregon Spur orchards, mites were found in all the mummies from these orchards (Table 3).

Tendency of *Tarsonemus* mites to be associated with the core and calyx of healthy and diseased mature apple fruits – *Determination of core rot diseases (WCR and DCR) and mites in the core and calyx of mature fruits* – DCR and WCR were found in fruits from almost all of the Red Delicious orchards. The incidence of DCR in these orchards ranged from 0.4% to 6%, and that of WCR from 0.0% to 1.7% (Table 1). In Granny Smith orchards, no DCR or WCR was found. However, a dry decay in the calyx tube (hereafter referred to as calyx tube decay) was observed at an incidence of 0.3–9.6% (Table 2). Calyx tube decay lesions resembled DCR lesions in that they consisted of dark brown, dry and firm lesions that sometimes penetrated the soft tissue surrounding the calyx tube to a depth of 1–2 mm (Fig. 2).

Tarsonemus mites were found in both Granny Smith and Red Delicious fruits. However, in Red Delicious fruits, mites were present in the core and calyx (Table 1), whereas in Granny Smith fruits, mites were restricted to the calyx tube (Table 2). In Red Delicious fruits the incidence of mites in the core of DCR fruits varied from 50% to 100% (average 83.90%), whereas in healthy fruits the incidence varied from 0% to 66.7% (average 56.0%). For Red Delicious WCR fruits the incidence of mites in the core varied from 0.0% to 100% (average 67.9%; Table 1).

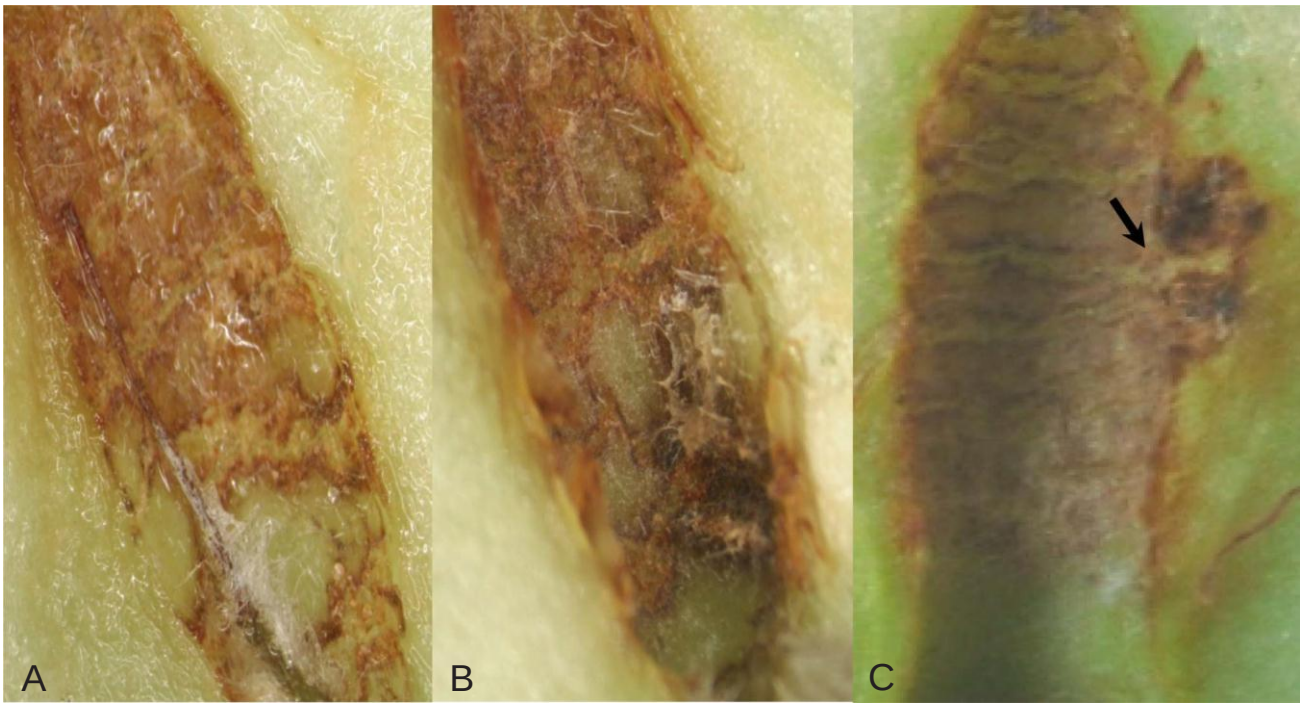

Fig. 2. Calyx tube of Granny Smith mature fruit showing – A. no symptoms; B. calyx tube decay symptom; C. calyx tube decay symptom associated with cracks (arrow) within the calyx tube.

In Granny Smith fruits with calyx tube decay the incidence of the mites in the calyx tube varied from 16.7% to 100% (average 52.8%), whereas in healthy fruits their incidence varied from 0% to 58.4% (average 48.9%; Table 2).

Statistical analyses – The results of the tests for independence are presented in Table 4. The development of DCR in Red Delicious fruits was dependent on the presence of mites in the core ($P < 0.0001$) but was inversely related to the presence of mites in the calyx. In Red Delicious fruits the development of WCR was independent of the presence of mites in the core ($P = 0.44$) and calyx tube ($P = 0.11$). Calyx tube decay in Granny Smith fruits was independent ($P = 0.72$) of mites in the calyx.

The ability of *Tarsonemus* mites to complete their life cycle on *Alternaria* sp., *Penicillium* sp., and *Cladosporium* sp. cultures – The *Alternaria* isolate obtained from a DCR lesion was stably transformed with the red fluorescent protein gene *DsRed-Express*. The transformant exhibited constitutive fluorescence in spores as well as mycelia when viewed using epifluorescence microscopy (Fig. 3 A). The level of expression of the protein was high, frequently resulting in pink-colored spores when viewed with a light microscope.

Temperature influenced the survival of the mites on *Penicillium* sp. and *Alternaria* sp. cultures. After 7 days, mite survival on plates incubated at 30°C was 100%, whereas <20% of the mites on plates incubated at 25°C survived. After 14 days of incubation only 10–20% of mites were viable on plates incubated at 30°C, whereas no live mites were detected on plates incubated at 25°C. No mites survived on the control PDA plates that were not inoculated with any fungus. *Tarsonemus* mites did not produce eggs or nymphal stages and were unable to complete their life cycle on *Penicillium* sp. or *Alternaria* sp. The same trend was observed for the repeat of the experiment.

Tarsonemus mites placed on the red fluorescent *Alternaria* cultures contained a light red to pink color when viewed under the stereomicroscope (Fig. 3B), indicating that they had ingested the fungus. Visualization of these mites using fluorescence microscopy revealed bright fluorescence within the gut of the mites, but no discernable fungal structures were observed (Fig. 3 C, D). Mites that were placed on untransformed *Alternaria* cultures did not contain a light red color nor did they show any fluorescence when viewed using epifluorescence microscopy.

Cladosporium sp. was a better food source for the mites than *Alternaria* sp. and *Penicillium* sp. Mites placed on *Cladosporium* sp. cultures that were incubated at 30°C for 7 days multiplied extensively and could not be counted accurately, increasing from 10 to

Table 4. Results of association analyses to determine if the presence of wet core rot, dry core rot, and calyx tube decay were dependent on the presence of mites in the calyx or core.

Association	*P*-value[a]		Percentage of healthy and diseased apples containing mites	
			No mites	Mites
Red Delicious apples				
DCR with mites in core	<0.0001	DCR	16	84
		Healthy	44	56
DCR with mites in calyx	<0.0001	DCR	98	2
		Healthy	83	17
WCR with mites in core	0.4401	WCR	32	68
		Healthy	40	60
WCR with mites in calyx	0.1061	WCR	96	4
		Healthy	85	15
Granny Smith apples				
Calyx tube decay with mites in calyx	0.7184	Calyx tube decay	47	53
		Healthy	51	49

Notes: [a]Using Fisher's exact test.
DCR, dry core rot; WCR, wet core rot.

more than 150 mites per plate. In contrast, plates that were incubated at 25°C only showed a two- to three-fold increase in mite numbers. Mites incubated for 14 days at 30°C increased to 250–300, whereas at 25°C only approximately 50 mites were observed per plate. The same trend was observed when the experiment was repeated.

DISCUSSION

Tarsonemus mites differentially colonized several important apple developmental stages (buds, blossoms, 4-cm diameter fruit, mature fruits, and mummies) during the growing season. At the start of the season, the high incidence of mites in mummies suggests that mites may overwinter here. Mites could then emerge from the mummies in spring in small numbers and colonize buds, blossoms, and developing fruit. The mites would preferentially leave the mummies, because overcrowding and exhaustion of their food source will occur when they become active and start multiplying in spring. The mummies are not the only overwintering source for the mites as some, but not all, Tarsonemid species can also overwinter in the soil in organic debris, moving upward with insects emerging from the soil, and migrating to the new growth in spring and summer (Lindquist, 1986). Apple fruits that remain on the orchard floor in winter have also been reported as being an overwintering refuge site for some mites including *Typhlodromus pyri* and *Typhlodromus occidentalis* (Gurr *et al*., 1997).

In mature fruits, the calyx tube and core appear to be ideal sites for *Tarsonemus* mites because colonization of these was high (up to 94%) in all orchards, although in Granny Smith orchards mites were only found in the calyx tube. The predominance of *Tarsonemus* mites in the core and calyx tubes may be because (1) these regions provide *Tarsonemus* mites with food and protection from predators and acaricides and (2) these mites are small (100–300 μm), which allows them to easily enter the calyx tube, which is relatively large (1800–2400 μm). As some of the other mite families identified in our study also have small size ranges (250–420 μm) (Zhang, 2003), the high incidence of *Tarsonemus* mites in fruits might rather be a result of their attraction to fungi growing in the core and calyx.

Tarsonemus mites are not the only mites that can potentially vector fungal spores to the core of mature fruits, because mites in the families Phytoseiidae, Tetranychidae, Tydeidae, and Dolichocybidae were also identified. However, these mites occurred at low frequencies (0–3.40%), and they are most likely of minor importance in the dispersal of core rot pathogens. The Phytoseiidae was most prevalent in the core, followed by the Tydeidae. Gurr *et al.* (1997) also reported that mites in the family Phytoseiidae occur within the calyx tube of apples in New South Wales. Phytoseiids are used for the biological control

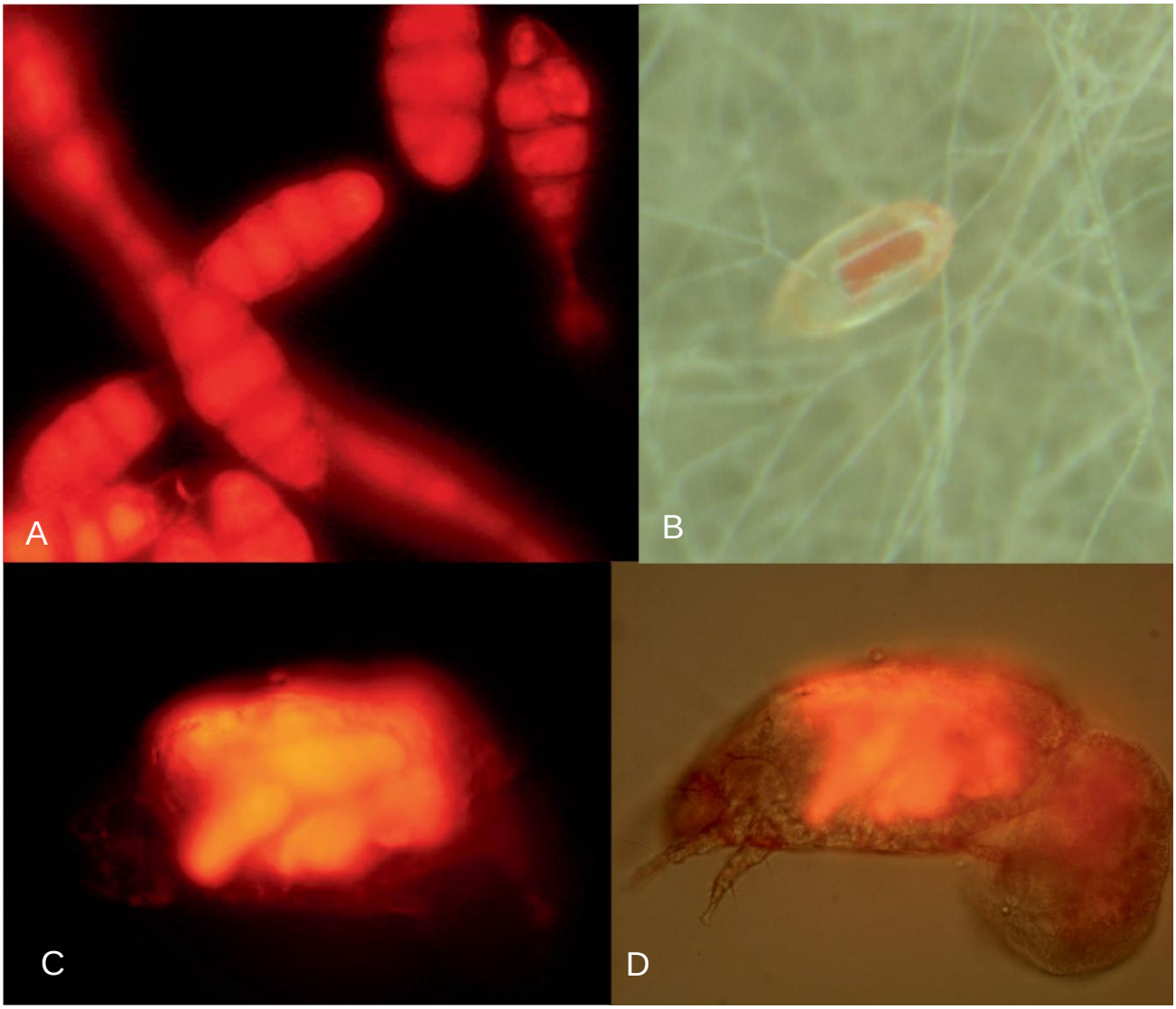

Fig. 3. A. Culture of an *Alternaria* sp. transformant expressing the *DsRed-Express* gene in conidia as viewed using epifluorescence microscopy; *Tarsonemus* mites that were fed on the *DsRed-Express Alternaria* sp. transformant as viewed using; B. light stereomicroscope; C, D. epifluorescence microscopy.

of phytophagous spider mites (Tetranychidae) (Gerson *et al*., 2003), and seem unlikely to vector fungal spores. In contrast, mites in the family Tydeidae are potential fungal vectors. Although they can cause injury to some crops, they are mainly omnivorous and feed on fungi as well as plant litter (Jeppson *et al*., 1975). They are known as cleaners and are always on the move, with fungal spores sticking to setae on their bodies as the larvae feed on fungi such as *Penicillium* and *Colletotrichum* (McCoy *et al*., 1969).

Analyses of core-rot-susceptible Red Delicious fruits for the presence of mites within diseased and healthy fruits showed a significant association of *Tarsonemus* mites in the core of fruits with DCR. This agrees with findings reported by Michailides *et al.* (1994), who also found a high incidence (50–86%) of *T. confusus* in diseased fruits. However, unlike Michailides *et al.* (1994), we also identified a high incidence of *Tarsonemus* mites in the core of healthy fruits. This suggests that if mites do play a role in disease development, several other factors may also be important in the determination of disease development. The lack of a significant association of *Tarsonemus* mites in the core of fruits with WCR may be because of

the low number of WCR fruits (only 28) found in the survey.

During the analyses of mature fruits for mites, a previously unreported calyx tube decay was observed in Granny Smith fruits, but no core rot symptoms. There was no significant association of mites with these calyx tube decay symptoms in Granny Smith fruits. The calyx tube decay symptom was observed at frequencies (0.33–9.68%) similar to those of DCR (0.4–6.0%) in Red Delicious fruits. The specific causative agent of the calyx tube decay could be an *Alternaria* sp., because the symptoms appeared similar to those of DCR. However, this would require further investigations because there are many other fungi that could be involved. The small, restricted size of the calyx tube decay lesion might be the result of a lower pH in mesoderm tissue just outside the core region of resistant cultivars (Niem *et al*., 2007).

In South Africa, the *Tarsonemus* mites in apple orchards may consist of three species, including *T. waitei* and two putative new species with closest similarity to *T. mixtus* and *T. bilobatus*, respectively. The identification of *Tarsonemus* species is challenging, since there are more than 200 described *Tarsonemus* spp. for which the taxonomy of several is still controversial (Lindquist, 1986). Therefore, more specimens will have to be collected from South African orchards before the specimens can be described as new species. It was clear from the mite species identifications that the *Tarsonemus* species that was identified in California, *Tarsonemus confuses* (Michailides *et al*., 1994), is not present in South African apple orchards.

The *Tarsonemus* mites associated with apples are most likely fungivorous. *Cladosporium* sp. was identified as one of the fungal genera that served as a food source for reproduction of the mites, whereas *Penicillium* sp. and *Alternaria* sp. were not. The fact that the mites ingested *Alternaria*, but could not reproduce or survive on these culture plates, may suggest that *Alternaria* is toxic to the mites. The mites, however, may still vector *Alternaria* spores while searching for other fungi such as *Cladosporium* spp. in the core region and flower parts of fruits. Support for this hypothesis comes from the fact that *Alternaria* spores were frequently observed in mummies, the flower parts of 4-cm diameter fruit, and mature fruit. The *Tarsonemus* spp. require relatively high temperatures for activity because they survived and reproduced better at 30°C than at 25°C. In general, the optimum temperature for Tarsonemid mite reproduction is 30°C, with the reproduction rate slowing down at temperatures below 20°C (Lindquist, 1986).

Our study provides preliminary evidence for a possible role of *Tarsonemus* mites in the epidemiology of apple core rot diseases. It will be important in future studies to conduct inoculation studies to determine if co-inoculation of core rot pathogenic fungi and *Tarsonemus* mites can increase disease incidence and severity. During the course of our research these studies could not be conducted because mite reproduction on pathogenic fungi did not yield a sufficient number of mites for inoculation experiments. Hence, conditions for reproduction of the mites must be optimized by further investigating the effect of temperature and different fungal species and genera on reproduction of the mites. Investigations will also have to determine whether the mites are strictly fungivorous, or whether they also feed on apple tissue, hence creating wounds and entry points for fungal pathogens.

ACKNOWLEDGMENTS

We thank the South African Apple and Pear Producers' Association (SAAPPA) and the National Research Foundation (NRF) of South Africa for funding this work. We also thank Dr. Ken Pringle (Department of Conservation Ecology and Entomology, Stellenbosch University) for useful discussions on *Tarsonemus* and predatory mites in apple orchards, and Prof. Gustaf Holz (Department of Plant Pathology, Stellenbosch University) for interesting discussions on the possible role of insects in the etiology of core rots. We are also grateful to Marieta van der Rijst (Agricultural Research Council, Biometry Unit, Stellenbosch, South Africa) for assistance with statistical analyses of the data and Bekker Wessels (ProCrop Trust, Ceres, South Africa) for the identification of research orchards and collection of material.

REFERENCES

Clewer, A. G. and D. H. Scarisbrick. 2006. Practical Statistics and Experimental Design for Plant and Crop Science. John Wiley and Sons, Chichester, UK.

Combrink, J. C. 1983. The etiology of apple core rot diseases. [PhD plant pathology]. University of Pretoria Press, Pretoria, South Africa.

Combrink, J. C., J. Coreen, C. J. Grobbelaar and T. R. Visagie. 1987. Effect of diphenylamine emulsifiable concentrates on the development of wet core rot in Starking apples. Decid. Fruit Grower 37: 97–99.

Combrink, J. C. and L. Ginsburg. 1973. Core rot in Starking apples – a preliminary investigation into

the origin and control. Decid. Fruit Grower 23: 16–19.

Combrink, J. C., J. M. Kotze and T. R. Visagie. 1985. Colonization of apples by fungi causing core rot. Hortic. Sci. 2: 9–13.

Combrink, J. C., J. M. Kotze, F. C. Wehner and C. J. Grobbelaar. 1985. Fungi associated with core rot of Starking apples in South Africa. Phytophylactica 17: 81–83.

Dowding, P. 1969. The dispersal and survival of spores of fungi causing bluestain in pine. Trans. Br. Mycol. Soc. 52: 125–137.

Ellis, M. A. and J. G. Barrat. 1983. Colonization of Delicious apple fruits by *Alternaria* spp. and effect of fungicide sprays on moldy-core. Plant Dis. 67: 150–152.

Gerson, U., R. L. Smiley and R. Ochoa. 2003. Mites (Acari) for Pest Control. Blackwell Science, Cambridge, UK.

Gurr, G. M., W. G. Thwaite, B. J. Valentine and H. I. Nicol. 1997. Factors affecting the presence of *Typhlodromus* spp. (Acarina: Phytoseiidae) in the calyx cavities of apple fruits and implications for integrated pest management. Exp. Appl. Acarol. 21: 357–364.

Hofstetter, R. W., J. Cronin, K. D. Klepzig, J. C. Moser and M. P. Ayres. 2006. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia 147: 679–691.

Jeppson, L. R., H. H. Keifer and E. W. Baker. 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, CA, USA.

Klepzig, K. D., J. C. Moser, F. J. Lombardero, R. W. Hofstetter and M. P. Ayres. 2001. Symbiosis and competition: Complex interactions among beetles, fungi and mites. Symbiosis 30: 83–96.

Lindquist, E. E. 1986. The world genera of Tarsonemidae (Acari: Heterostigmata): A morphological, phylogenetic, and systematic revision, with a reclassification of family-group taxa in the Heterostigmata. Mem. Entomol. Soc. Can. 136: 1–517.

Lombardero, M. J., M. P. Ayres, M. W. Hofstetter, M. C. Moser and K. D. Klepzig. 2003. Strong indirect interactions of *Tarsonemus* mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Oikos 102: 243–252.

McCoy, C. W., A. G. Selhime and R. F. Kanavel. 1969. The feeding behavior and biology of *Parapronematus acacia* (Acarina: Tydeidae). Fla. Entomol. 52: 13–19.

McDaniel, B. 1979. How to Know the Mites and Ticks. The Pictured Key Nature Series. South Dakota State University, Wm. C. Brown Publishers, Dubuque, IA, USA.

McLean, T., P. H. Fourie and A. McLeod. 2009. Reporter gene transformation of the trunk disease pathogen. *Phaeomoniella chlamydospora* and biological control agent *Trichoderma harzianum*. Australas. Plant Pathol. 38: 153–167.

McLeod, A., F. J. Smit, L. Van der Walt and L. Mostert. 2008. Apple core rot diseases past and present. South Afr. Fruit J. 7: 21–24.

Michailides, T. J., D. P. Morgan, E. Mitcham and C. H. Crisosto. 1994. Occurrence of moldy core and core rot of Fuji apple in California. Cen. Val. Postharvest Newslett. 3: 6–9. [Cooperative extension, University of California, Kearney Agricultural Centre, Parlier, CA, USA].

Miller, P. M. 1959. Open calyx tubes as a factor contributing to carpel discoloration and decay of apples. Phytopathology 49: 520–523.

Moser, J. C. 1976. Surveying mites (Acarina) phoretic on the southern pine beetle (Coleoptera: Scolytidae) with sticky traps. Can. Entomol. 108: 809–813.

Moser, J. C., T. J. Perry, J. R. Bridges and H. F. Yin. 1995. Ascospore dispersal of *Ceratocystiopsis ranaculosus*, a mycangial fungus of the southern pine beetle. Mycologia 87: 84–86.

Niem, J., I. Miyara, Y. Ettedgui, M. Reuveni, M. Flaishman and D. Prusky. 2007. Core rot development in Red Delicious apples is affected by susceptibility of the seed locule to *Alternaria alternata* colonization. Phytopathology 97: 1414–1421.

Roets, F., P. W. Crous, M. J. Wingfield and L. L. Dreyer. 2009. Mite-mediated hyperphoretic dispersal of *Ophiostoma* spp. from the infrutescences of South African *Protea* spp. Environ. Entomol. 35: 143–152.

Roets, F., M. J. Wingfield, P. W. Crous and L. L. Dreyer. 2007. Discovery of fungus– mite mutualism in a unique niche. Environ. Entomol. 36: 1226–1237.

Serdani, M., P. W. Crous, G. Holz and O. Petrini. 1998. Endophytic fungi associated with core rot of apples in South Africa, with specific reference to *Alternaria* species. Sydowia 50: 257–271.

Serdani, M., J. C. Kang, B. Andersen and P. W. Crous. 2002. Characterization of *Alternaria* species-groups associated with core rot of apples in South Africa. Mycol. Res. 106: 561–569.

Spotts, R. A. 1990. Moldy core and core rot. pp. 29–30. In: Jones, A. L. and H. S. Aldwinckle (Eds.). Compendium of Apple and Pear Diseases. The American Phytopathological Society, St Paul, MN, USA.

Spotts, R. A., R. J. Holmes and W. S. Washington. 1988. Factors affecting wet core rot of apples. Australas. Plant Pathol. 17: 53–57.

Teixido, N., J. Usall, N. Magan and I. Vinãs. 1999. Microbial population dynamics on Golden Delicious apples from bud to harvest and effect of fungicide applications. Ann. Appl. Biol. 134: 109–116.

Van der Walt, L. 2009. Characterization of mites and *Penicillium* species associated with apple core rot diseases [MSc plant pathology]. Stellenbosch University, South Africa.

Van der Walt, L., R. A. Spotts, C. M. Visagie, F. J. Smit and A. McLeod. 2010. *Penicillium* species associated with preharvest wet core rot in South Africa and their pathogenicity on apple. Plant Dis. 94: 666–675.

Zhang, Z.-Q. 2003. Mites of Greenhouses. Identification, Biology and Control. CAB International, Cambridge, UK.