An Overview of Research on Diseases of Cactus Pear in South Africa♦

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ABSTRACT

Since 1996, the primary objective of the New Crop Pathology Program at the University of the Free State has been to conduct a systematic survey of diseases occurring in cactus-pear [*Opuntia ficus-indica* (L.) Miller] orchards throughout the country and to investigate possible relationships between disease-causing microorganisms and various insects, specifically *Drosophila* sp. Numerous fungal genera, of which the most prominent were *Alternaria*, *Rhizopus*, and *Fusariu,* were isolated from the exterior tissue layer of nonsterilized fruit. Two genera, *Rhizopus* and *Penicillium*, were isolated at frequencies of 34% and 10%, respectively, from rotting fruit. *Alternaria tenuissima* has been isolated from symptoms on cladodes that include small, superficial, chlorotic spots on the cuticle, which coalesce to form raised grey scabs. *Lasiodiplodia theobromae* (teleomorph: *Botryosphaeria rhodina*) has been isolated from roundish, black cankers on cladodes characterized by black gum exudation from the perimeter of the canker. A clear association between *Drosophila* species and mycelial fungi was identified.

INTRODUCTION

The utilization of fruit from wild cactus-pear [*Opuntia ficus-indica* (L.) Miller] stands in South Africa has been ongoing for hundreds of years. Commercial cultivation of cactus pear in South Africa is, however, a recent undertaking (Brutsch, 1984; 1997). Much of the developing cactus-pear industry in South Africa is based on spineless cultivars of *Opuntia ficus-indica* introduced into the country during 1914 (Brutsch and Zimmermann, 1993). Some of these included the various Burbank spineless varieties, primarily utilized for fodder.

Today, it is estimated that South Africa has approximately 1,500 hectares of cactus pear under intensive cultivation, which yield about 15,000 tonnes. The need for research on cactus-pear diseases in South Africa became evident six years ago after local growers had increasingly experienced disease-related problems.

Few systematic studies have focused on diseases of cactus pear anywhere in the world (Granata, 1995; Granata and Sidoti, 2000). To date, only four fungal pathogens have been formally reported on the genus *Opuntia* in South Africa. The fungus*, Didymosphaeria opulenta* (De Not.) Sacc. was first reported on the genus *Opuntia* in South Africa but the report is from *Opuntia stricta* Haw., and not *O. ficus-indica* (Crous

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et al., 2000). More recently, three fungal pathogens associated with diseases of cladodes of *O. ficus-indica* were reported (Swart and Kriel, 2002).

Currently, the only research done on diseases of *O. ficus-indica* is being coordinated by the New Crop Pathology Program (NCPP) established at the University of the Free State in Bloemfontein, South Africa. Since 1996, the NCPP has been conducting the first formal investigation in South Africa of diseases associated with *O. ficus-indica*. The objective of the NCPP's investigations thus far has been to conduct a systematic survey of diseases occurring in cactus-pear orchards throughout the country and the possible relationships between disease-causing microorganisms and various insects, specifically *Drosophila* sp.

Research conducted in South Africa thus far has identified numerous fungal and bacterial pathogens, which have been shown to cause diseases of *O. ficus-indica* in other parts of the world (Farr *et al.*, 1989; Fucikovsky 1990; Granata, 1995; Granata and Sidoti, 2000). New fungal associations of cactus pear, not yet recorded in other parts of the world, have also been identified. This review will highlight and discuss some of the most important findings in this regard.

POSTHARVEST DISEASES

One of our earlier investigations on cactus-pear fruit was to identify fungi that are naturally associated with healthy fruit as a holistic basis on which to conduct future research of fruit diseases. Isolations of fungi from areoles and the peel tissue, without prior disinfecting, were conducted on agar media containing streptomycin sulphate (0.33 g/1000 ml). Subsequent experiments also investigated the mycoflora of fruit that had been exposed to two different levels of superficial sterilization with NaOCl. At least 13 genera of fungi (Table 1), of which the most prominent were *Alternaria*, *Rhizopus*, and *Fusarium*, were isolated from the exterior tissue layer of nonsterilized fruit. The number of fungal colonies and taxa decreased with increasing degrees of sterilization, but *Alternaria* sp. was, again, the most prominent fungal taxon isolated from areoles.

A thorough investigation of soft rot of cactus-pear fruit cultivars Algerian and Gymno Carpo following harvest was also conducted. Symptomatic fruit was soft and oozed a red, sticky exudate. No unpleasant smell was present, which suggested that bacteria and yeast were not responsible. A certain amount of fermentation of fruits was evident because many fruits had become distended to the point of almost bursting. Efforts were made to isolate the microorganism(s) responsible for the rot. The exudate from symptomatic fruits was streaked onto Petri plates containing malt extract agar (MEA) in an attempt to isolate fungi and bacteria.

No attempt was made in this first attempt to isolate yeasts, but in a second experiment, nutrient agar (NA) for isolating yeasts was used. Only two fungi, *Rhizopus* sp. and *Penicillium* sp. were isolated at frequencies of 34% and 10%, respectively. Bacterial isolates comprised 5% of isolations and 51% of plates yielded no colonies. Isolations conducted on NA yielded four species of yeast, *Haenseniaspora ovarum* (66%), *Pichia kluyveri* (29%), *P. membranafaciens* (3%) and *Candida* sp. (2%), but because no pathogenicity studies were conducted on healthy fruit, their role in causing rot of cactus-pear fruit remains to be investigated. Two species of yeast, *Pichia heediii* and *P. cactophila* have been associated with decaying tissue of cacti in North America (Phaff *et al.*, 1978; Starmer et al.,1978).

These above findings are very significant as far as the postharvest handling of cactus-pear fruit is concerned. The persistence of fungal propagules in areoles, and in the case of *Alternaria* sp., in the skin of cactus fruits, despite intensive superficial disinfestation of fruit is an important factor that must be taken into account when considering ways to prevent rotting of fruit during storage and transportation. The fact that fungal inoculum is present on newly harvested fruit requires that careful attention is paid to the packing procedure of fruit so as not to wound or bruise fruit through which infection by resident fungi can take place. This, together with the influence of the physical/abiotic environment on physiological changes within plants (Lakshminarayana and Estrella, 1978; Berry and Nobel, 1985; Nerd and Nobel, 1991) and how these may be linked to the onset of fruit rot, is, therefore, in need of urgent investigation. The correct storage of fruit at temperatures low enough to not cause "chilling injury" or high enough to allow saprophytic fungal growth is also an important consideration.

CLADODE DISEASES

Symptoms of cladode diseases are difficult to attribute to a specific pathogen because disease complexes between fungi and bacteria may be common in cacti owing to their physiology (Nerd and Nobel, 1991). Furthermore, the role of abiotic factors in either predisposing cacti plants to infection, or in exacerbating infection is unknown and can further complicate diagnosis, The inference can thus be drawn that many factors related to host physiology and the environment can trigger disease development in cactus pear. The biochemical characteristics of cacti generally are such that the evolution of disease is very rapid because of the large amount of moisture and high concentration of sugars in cactus tissue (Berry and Nobel, 1985; Nerd and Nobel, 1991). This provides for an ideal environment and easily accessible source of nutrients for microorganisms. The situation is exacerbated by the fact that insects attracted to the sweet exudations of rotting fruit transmit the reproductive propagules of fungal and bacterial pathogens to healthy plants.

In Mexico, the world's largest producer of cactus pear, intensive monoculture of cactus pear has resulted in the appearance of numerous pest and disease problems (Fucikovsky, 1990). Approximately 122 species of insects are presently known to be associated with *Opuntia* spp. (Longo and Rapisarda, 1995) while numerous species of pathogenic fungi and bacteria have also been recorded (Farr *et al.*, 1989; Granata, 1995). Diseases of cladodes that have been investigated in South Africa during the last five years have revealed the presence of numerous fungi. *Alternaria tenuissima* has been isolated from a dry superficial necrosis of the cuticle and underlying tissue up to 3 mm in depth. Symptoms include small chlorotic spots on the cuticle, which coalesce to form raised grey scabs. A *Fusarium,* hitherto unidentified*,* has been isolated more commonly from dry necrotic lesions that were darker, larger, and less superficial, sometimes extending through the tissue to the opposite side of the cladode.

Lasiodiplodia theobromae (teleomorph: *Botryosphaeria rhodina*) has been isolated from roundish, black cankers (15 mm to 50 mm in diameter) on cladodes characterized by black gum exudation from the perimeter of the canker. Pycnidia are often evident on the surface of the canker. Pathogenicity test demonstrated the aggressiveness of these three species of fungi in colonizing cladodes following artificial inoculations in the glasshouse. Mean lesion diameters measuring 15, 27, and 44 mm for *A. tenuissima*, *Fusarium* sp., and *L. theobromae*, respectively, were recorded 14 days after inserting wooden toothpick tips that had been colonized by the pathogens into each of five cladodes of 18-month-old potted plants of *O. ficus-indica* (cv Morado). *Alternaria* sp. and *Botryosphaeria rhodina* have been reported on *Opuntia* sp. in the United States (2) but no records of the above three fungi occurring on *O. ficus-indica* were found. *Alternaria alternata* (Fries : Fries) von Keissler has been associated with the symptom known as "golden spot" in Italy (Granata and Sidoti, 1997). Although numerous other fungi having known pathogenic potential have been isolated from diseased cladodes in South Africa, their pathogenic status has not yet been determined.

Digital photographs are regularly taken of cladode disease symptoms and integrated with data pertaining to possible causal agents and contributing environmental factors. The information is then incorporated into the *Opuntia* disease database maintained by the NCPP in Bloemfontein. Microorganisms are kept in a central culture collection, which is unique in South Africa and a great asset to local cactus-pear growers.

The advantage of this is that detailed studies of cladode diseases can now be conducted as well as the screening of *O. ficus-indica* cultivars for disease resistance.

INSECT-FUNGAL ASSOCIATIONS

There are numerous reports of insects, such as flies, acting as vectors for microorganisms that can cause disease in *Opuntia* sp. (Harris and Maramarosch, 1980). The families Syrphidae, Otitidae and Ephydridae have been shown to be vectors of *Erwinia carotovora* subsp. *carotovora* the causal agent of cladode soft rot (Fucikovsky, 1990; Varvaro *et al*., 1993). *Drosophila* spp. have been associated with the dispersal of numerous fungal pathogens that cause disease on other plants (Barker and Starmer, 1982; Lack, 1989; Michailides and Spotts, 1990; Louis *et al.*, 1996; Hodge *et al.*, 1997). Vinegar flies are commonly found around fallen fruit in cactus-pear orchards, and their potential as vectors of fungal propagules is obvious. *Drosophila* larvae and adults feed on fungi and bacteria in decaying cactus-pear fruits, which serve as a rich substrate for microorganisms.

Our studies have demonstrated a clear association between *Drosophila* species and many fungi that are potentially pathogenic to cactus-pear fruit (Table 1). Overall, 13 genera of mycelial fungi were identified from *Drosophila* sp. in South Africa of which *Mucor* sp. (43.3%; 32.0%) and *Fusarium* sp. (16.1%; 7.7%) were most prominent from *D. melanogaster* and *D. hydei*, respectively. Yeasts represented 22.0% and 20.4% of isolations from *D. melanogaster* and *D. hydei*, respectively. Pathogenicity tests were positive for all isolates tested by means of artificial inoculation of cladodes using toothpicks infested with the respective pathogens. *Microdochium* sp.2 and *Alternaria tenuissina* formed significantly ($P<0.05$) larger lesions than the other fungi (Table 1). Koch's postulates were confirmed by the re-isolation and identification of all artificially inoculated fungi.

Table 1. Fungi isolated from individual specimens of *Drosophila melanogaster* and *D. hydei*
Mean lesion diameters caused by fungal isolates on cladodes of *Opuntia ficus-indica*;
control treatment was 9.72 mm.

Fungi Isolated	Percentage Recovery		Mean Lesion Length (mm)*
	D. melanogaster	***D. hydei***	
Arthrographis. sp.	1.7	6.8	-
Alternaria tenuissima	0.0	4.9	15.22 c
Aspergillus niger	0.9	2.9	12.82 b
Aschochyta sp.	0.0	1.0	12.74 b
Aureobasidium sp.	0.0	1.0	-
Cladosporium sp.	4.2	0.0	-
Fusarium proliferatum	2.9	3.5	13.74 b
Fusarium verticilloides	10	1.5	13.30 b
Michrodochium sp1	2.2	1.2	12.92 b
Michrodochium sp2.	1.0	1.5	19.99 d
Mucor sp. 1	8.5	6.9	12.34 b
Mucor sp. 2	9.3	6.9	12.53 b
Mucor sp. 3	14.4	8.7	12.60 b
Mucor sp. 4	11	9.7	12.61 b
Paecilomyces sp.	0.0	2.9	-

Fungi Isolated	Percentage Recovery		Mean Lesion Length (mm)*
	D. melanogaster	*D. hydei*	
Penicillium sp.	3.4	9.7	-
Phoma sp.	2.5	6.8	12.60 b
Trichoderma sp.	0.0	1.0	-
Yeasts	22.0	20.4	-
Unidentified fungi	5.9	2.9	-
Control	?	?	?

*Values followed by different lower case letters are significantly different at P<0.05

CONCLUSIONS

A firm foundation has been laid by the NCPP at the University of the Free State for research into cactus-pear diseases in South Africa. Preliminary findings emphasize the need for an integrated approach to managing diseases of cactus pear in South Africa. In terms of this objective, the focus would have to be on understanding all biotic and abiotic sources of stress that can affect the health and productivity of cactus-pear plantations. Within this approach of holistic plant health management emphasis will be placed on the interaction of all forms of stress rather than on any single source of stress. It is very important that research on all aspects of cactus-pear cultivation is expanded if the industry in South Africa is to grow at the current pace.

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