preliminary test, six three-month-old plants of each clone were inoculated with a bacterial suspension. Control plants were inoculated with sterile water. The inoculation technique used involved carefully removing the seedlings from the growth media, clipping the root tips and dipping them in a bacterial suspension. In the case of clone GC550, wilting was evident after three days. Clones GC515 and GC505 appeared to be less susceptible with fewer plants showing signs of disease. A rapid screening technique to detect *R. solanacearum* will be developed in the near future and clones will be tested to determine their level of tolerance to this bacterium.

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

M. Serdani, P.W. Crous and G. Holz

*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa.*

Core rot of Red Delicious-type apple cultivars is a serious post-harvest disease in South Africa, and has been associated with losses of between 5% and 8%. Several facultative parasitic fungi have been isolated from diseased apples, of which *Alternaria alternata* (Fr.:Fr.) Kiessl. has proved to be the most dominant. Core rot symptoms have been detected as early as 3–6 weeks before harvesting, suggesting early infection. The aims of the present study were to determine which fungi are associated with the disease, establish the time of fruit colonisation, and characterise the *Alternaria* complex associated with the disease. Apple fruit were sampled for fungal endophytes at five different stages of fruit development, namely, bud stage, full bloom, just after fruit set, fruit 4 cm in diameter, and mature fruit. The cultivars Top Red (susceptible to core rot) and Granny Smith (resistant to core rot) were sampled during the 1995/96 growing season. Of the 40 different fungal taxa encountered, 18 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. As found in previous studies, the *Alternaria* complex was the most dominant, representing 57% of the total number of 1602 isolates. However, based on sporulation patterns and spore morphology, this complex could be divided into three different groups in the *Alternaria alternata*, and two in the *Alternaria infectoria* E.G. Simmons complexes. A further group was identified which may represent an additional *Alternaria* species. This study has also shown that the *Alternaria* spp. are already present as endophytes at the bud development stage, which has serious implications for any programme using fungicides for disease control.

## Renewed interest in *Botryosphaeria* on Proteaceae

S. Denman[1], P.W. Crous[1] and M.J. Wingfield[2]

*[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa; [2]Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa.*

*Botryosphaeria* species have been associated with Proteaceae in South Africa for many years, but were considered weak or secondary pathogens. Recently, *Botryosphaeria* has been isolated consistently from commercially propagated *Protea magnifica* and *Protea repens* plants with severe canker disease. On both of these hosts stem cankers were the most common disease symptom found. The initial stage of disease development on *P. magnifica* was indicated by necrosis of a single leaf on a stem. In advanced stages of the disease the dead leaves were retained on perished branches. In contrast, infection of *P. repens* was characterised by leaf drop in the cankerous zones on the stems. Symptoms on *P. grandiceps* appeared to be associated with insect damage. These observations show that host physiology is intimately involved with disease expression and might play an important role in disease epidemiology. Based on the cultural characteristics and general morphology of the fungi isolated, a number of different species of *Botryosphaeria* appear to be associated with the various symptoms displayed by diseased Proteaceae. In some instances more than one species was isolated from hosts such as *P. magnifica* and *P. repens*. The different species associated with distinct disease symptoms emphasises a need for further studies on the taxonomy and pathogenicity of these fungi. Knowledge of the epidemiology of the various *Botryosphaeria* diseases will also assist in the implementation of control strategies. In view of the current expansion in production and export of Proteaceae cut-flowers, information gained from these studies will greatly benefit this growing industry.

## Preliminary study of pythiaceous fungi associated with citrus root rot in South Africa

B. Maseko, T.A. Coutinho and M.J. Wingfield

*Tree Pathology Co-operative Programme, Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa.*

Production of citrus fruit for both the local and international markets is an important industry in South Africa. Pythiaceous fungi, especially *Phytophthora* spp., cause diseases of citrus in nurseries and orchards. These fungi infect roots and cause fibrous root rot, especially on susceptible rootstocks in orchards, resulting in loss of vigour and yield reduction. The role of *Pythuim* spp. in the development of the above symptoms remains unclear, despite the fact that these fungi are abundant in the rhizosphere of both healthy and diseased citrus trees. The aim of this study was to conduct a preliminary investigation on *Phytophthora* and *Pythium* spp. associated with diseased citrus trees. Samples of soil and diseased plant material were collected from citrus orchards and nurseries. More than three hundred *Phytophthora* and *Pythium* isolates were collected. The most abundant species were *Phytophthora nicotianae* and *Pythium irregulare*. A rapid screening technique to determine the virulence of the isolates was developed. Those isolates found to be virulent were further tested by inoculating Rough lemon and Troyer citrange seedlings. All *Phytophthora* isolates were pathogenic whereas *Pythium* isolates were either avirulent or only weakly pathogenic. These preliminary results will now be confirmed in further greenhouse trials.

## Effect of osmotic stress on growth and protease secretion by *Pseudomonas fluorescens*

R.J. Gwenzi[1], A.M. Clarke[1], J.P. Mildenhall[1] and B.A. Prior[2]

*[1]Department of Biochemistry and Microbiology, University of Fort Hare, Alice, 5700; [2]Department of Microbiology, University of the Orange Free State, Bloemfontein, 9300 South Africa.*

The growth of *Pseudomonas fluorescens* strain PSE in glucose yeast salts medium was measured at various water activity ($a_w$) values (0.998, 0.990, 0.980, 0.970, 0.960, 0.950), using NaCl as the osmoticum. Growth occurred at all $a_w$ values except 0.950. Total inhibition of growth would occur between 0.960 and 0.950 $a_w$. Lag phases of 2, 4 and 6 hours were observed at 0.980, 0.970 and 0.960 $a_w$, respectively. No lag phases were observed at 0.990 $a_w$ and in the control (0.998 $a_w$). The specific growth rate decreased from 0.35 (control) to 0.04 (0.960 $a_w$). Osmotic stress also affected extracellular protease for about 5 hours. Thereafter, production of extracellular protease resumed at a slower rate than the control. Maximum protease activity was obtained in the stationary phase of growth. At 0.960 $a_w$, the production of extracellular protease was inhibited. Intracellular protease activity was not detected in *Ps. fluorescens* PSE in the control or osmotically stressed media. *Ps. fluorescens* is an important food spoilage organism because it secretes enzymes, notably proteases, which degrade many food products.

## Identification of fungi associated with *Hypolixus harens* in *Amaranthus hybridus* stems

J.T. Blodgett[1], W.J. Swart[1], F.J. Kloppers[1] and S. v.d.M. Louw[2]

*Departments of [1]Plant Pathology and [2]Zoology and Entomology, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa.*

A sustainable pest and disease management approach for the cultivation of *Amaranthus hybridus* requires an understanding of abiotic and biotic

* factors affecting this potentially important food crop. Tissue discoloration and decay in stems and root collars of *A. hybridus* were observed in larval galleries of the pigweed weevil (*Hypolixus harens*). The objective of this study was to identify possible fungal species associated with this occurrence. Six-month-old *A. hybridus* stems were collected from two experimental plots in Bloemfontein, Free State, in 1997. Stems/root collars were split and small samples of discoloured tissue adjacent to insect galleries were aseptically transferred to corn-meal agar. Larvae were extracted from the stems/root collars and plated directly on cornmeal agar. Fungal isolates were transferred to half-plates of carnation-leaf agar/potato dextrose agar for identification. The most common species isolated from both the discoloured tissues and larvae was *Fusarium subglutinans* (58% and 54% of the samples, respectively). Other common species isolated from stems included other *Fusarium* spp. (10%), *Phomopsis* sp. (10%), and *Alternaria alternata* (10%). Other species isolated from the larvae included *F. equiseti* (11%) and other *Fusarium* spp. (11%), *Phomopsis* sp. (6%), and *A. alternata* (3%). The potential for significant disease loss associated with this insect–fungal association warrants further investigation.

## Timing of infection of pears by *Botrytis cinerea*

C.L. Lennox[1], R.A. Spotts[1] and G. Holz[2]

*[1]Mid-Columbia Agricultural Research and Extension Center, Oregon State University, 3005 Experiment Station Drive, Hood River, Oregon 97031, USA; [2]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa.*

Studies have indicated that *Botrytis cinerea* is the major pathogen causing post-harvest decay of the winter pear cultivar d'Anjou in the Hood River area of the US and that in this cultivar stem-end infections cause significantly higher levels of decay than calyx-end infections. It is widely assumed that infections resulting in calyx-end decay occur at or around full bloom. Consequently, measures aimed at controlling calyx-end rot caused by *Botrytis* involve the use of fungicides shortly before to shortly after full bloom. In order to determine when calyx-end infections occur, fruit from trees not sprayed with fungicides were collected every two weeks from full bloom until harvest, and the stamens, pistils and sepals were plated-out on an agar medium semi-selective for *Botrytis cinerea*. Results indicate that calyx infections have two distinct peaks, the main one being in mid- to late May, i.e. about a month after full bloom, and another much smaller one just prior to harvest. Similar results were obtained from isolations made from the cultivar Packham's Triumph in South Africa. Thus the application of an effective fungicide to the blossoms near full bloom would protect them from infection in the orchard and should significantly reduce the level of calyx-end decay occurring in storage.

## Patchy stunting of wheat and barley in Tanzania — preliminary results

K. van Dyk, J.J. du T. Meiring and L. Meyer

*Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002 South Africa.*

Patchy stunting (PS) poses a serious threat to dryland cultivation of wheat and barley in three major production areas of Tanzania, i.e. Hanang, West Kilimanjaro and Karatu. Estimated yield losses amount to approximately 5–30%, but could be as high as 40–50% during drier years on newly broken land. A survey was conducted to determine the incidence of the PS pathogen (*Rhizoctonia solani* AG-6) and other pathogenic fungi in the two main production areas and different soil types. A number of potentially pathogenic fungal species were found to occur in the roots and crowns of wheat and barley plants. Organisms commonly isolated include: *Bipolaris sorokiniana, Fusarium equisetii, F. nygamai, F. oxysporum, F. solani, Macrophomina phaseolina* and *Rhizoctonia* spp. Of these, *B. sorokiniana* and *F. nygamai* were most prominent. Preliminary results indicate no significant difference in organisms isolated from the different soil types or production areas. Contrary to the South African situation, PS occurs not only in the predominant black montmorillonitic clay soils, but also in clay, clay loam and sandy clay soils. However, the disease is more profound in the former soil type. Significantly higher Ca, Mg and Na levels in the black clay soils seem to have an effect on the growth of *R. solani*.

## Studies on the behaviour of *Botrytis cinerea* on pear using monoclonal antibody BC-KH4

C.L. Lennox[1], F.M. Dewey[2], R.A. Spotts[1] and G. Holz[3]

*[1]Mid-Columbia Agricultural Research and Extension Center, Oregon State University, 3005 Experiment Station Drive, Hood River, Oregon 97031, USA; [2]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK; [3]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa.*

Immunofluorescence microscopy using *Botrytis*-specific monoclonal antibody BC-KH4 is a useful tool for studying the behaviour of *Botrytis cinerea* on non-wounded and wounded pear fruit under various moisture regimes (i.e. as dry spores on dry fruit, as dry spores on fruit covered with a thin film of moisture and in droplets of inoculum). Tissue samples were processed for immunofluorescence 72 hours after inoculation. Ungerminated and germinated conidia showed antibody labelling, as did walls of germtubes and infection structures. Moisture encouraged growth on the fruit surfaces, and even without free water *Botrytis* was able to penetrate the skin directly or by means of simple appressoria. In both moisture regimes where water was present, a fluorescent fibrillar-like matrix was seen radiating from the germtube periphery, firmly attaching the germlings to the fruit surface. Non-wounded fruit did not decay, confirming previous reports that *Botrytis* requires a wound to cause decay in harvest ripe fruit. Under all moisture regimes germtubes of conidia deposited near wound sites were longer than those on non-wounded fruit. However, there was no indication of germtube growth towards the wound. Colonisation of lenticels under moist conditions and penetration by direct means or appressorium formation under all moisture regimes indicate that *Botrytis* is able to establish latent infections which only result in decay once the fruit surface is wounded. From this study it can be concluded that *Botrytis* spores present on pear fruit surfaces at harvest are an important source of inoculum for post-harvest decay and that control strategies must be aimed at limiting the amount and efficacy of this inoculum.

## Bacterial diseases of sunflower in South Africa

D. Fourie, A. Viljoen and P.S. van Wyk

*ARC-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520 South Africa.*

Three bacterial diseases are associated with sunflower in South Africa: apical chlorosis caused by *Pseudomonas syringae* pv. *tagetis*, bacterial leaf spot caused by *P. syringae* pv. *helianthi*, and bacterial stalk and head rot caused by *Erwinia carotovora* subsp. *carotovora*. Bacterial leaf spot is widespread and present under most environmental conditions, while apical chlorosis and bacterial stalk and head rot occur sporadically on plants injured by hail or insects. Bacterial stalk rot is the most damaging of the three diseases, sometimes leading to severe lodging in sunflower fields. Apical chlorosis occurs on sunflower plants before flowering only, and symptoms usually disappear within weeks. No control measures are being applied against any of the bacterial diseases, but field observations indicate that sources of resistance against bacterial leaf spot and bacterial stalk rot do exist.

## A survey of cucurbit viruses in KwaZulu-Natal

K.R. Cradock, J.V. da Graca and M.D. Laing

*Department of Microbiology and Plant Pathology, University of Natal, Scottsville, Pietermaritzburg, 3209 South Africa.*

A field survey of four important aphid-borne viruses of the Cucurbitaceae, zucchini yellow mosaic potyvirus (ZYMV), watermelon