CHAPTER 1

GENERAL INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are among the major food legumes in the world, and are grown on all the continents, except for Antarctica (Singh 1999). Beans represent an important source of protein, B-complex vitamins and minerals (Paradez-López *et al.* 1986) and form the staple food in the diets of many countries (De León *et al.* 1992). World production during 1997 amounted to 11 607 000 mt, produced on an area of 14 302 000 ha (Singh 1999). In South Africa, mean production of 58 000 t on 56 000 ha has been recorded, for the past 10 years (Coetzee 2000). Per capita consumption, in central and eastern Africa, exceeds 40 kg per annum (Singh 1999).

Bacterial diseases are commonly associated with dry beans wherever they are grown and often cause severe yield and seed quality loss (Allen *et al.* 1998). Three major bacterial diseases, common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli)* (Smith) Vauterin *et al.*, halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) (Burkholder) Gardan *et al.* and bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), van Hall, occur in South Africa. They are widely distributed throughout the bean producing areas (Fourie 2002), but incidence and severity vary annually as a result of biological and climatic factors and management practices.

Bacterial diseases affect foliage, stems, pods and seeds of beans (Yoshii 1980). Common bacterial blight leaf symptoms initially appear as water-soaked spots on the abaxial sides of leaves, which gradually enlarge, become flaccid and later turn brown and necrotic (Yoshii 1980, Saettler 1991). Lesions are often surrounded by a narrow

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zone of lemon-yellow tissue (Fig. 1). Pod lesions are water-soaked spots which gradually enlarge, turn red-brown and are slightly sunken (Fig. 2) (Yoshii 1980, Saettler 1991). Lesions usually vary in size and shape, and are frequently covered with bacterial ooze (Saettler 1991). Infected seeds are shrivelled and exhibit poor germination and vigour (Saettler 1991).

Halo blight leaf symptoms initially appear as water-soaked spots that later turn red-brown and necrotic. A lime-green halo frequently develops around the necrotic lesion (Fig. 3) (Schwartz 1989). Symptoms without halos may occur at temperatures exceeding 28°C. Jensen & Livingston (1944), however, identified isolates that produced halo-less lesions at 16°C. Stems may become infected and produce typical greasy spots. Pod symptoms are water-soaked, greasy spots that vary in size and may develop brown margins as they mature (Fig. 4). Infected seeds may rot or appear shriveled and discolored (Schwartz 1989). Internally-infected seed, however, exhibit few symptoms or are symptom-less (Taylor *et al.* 1979). Systemically infected plants exhibit a general lime-green color and plants are often stunted and distorted (Fig. 5) (Allen *et al.* 1998). Systemic chlorosis is more pronounced and uniform at temperatures below 20°C.

Bacterial brown spot leaf symptoms are small, irregular necrotic lesions that are sometimes surrounded by a narrow, pale green chlorotic zone (Fig. 6). Lesions may coalesce, dry out and become brittle, giving leaves a tattered appearance (Watson 1980). Pod lesions are small, dark-brown and deeply sunken, and may serve as a source of infection for seeds. Young infected pods may bend at the point of infection (Fig. 7) (Serfontein 1994).

Effective and economical control of bacterial diseases can only be achieved

using an integrated approach, including cultural practices, chemical sprays and genetic resistance. Planting of pathogen-free seed is the most important primary control method (Gilbertson *et al.* 1990), however, it does not guarantee disease control (Allen *et al.* 1998). Additional cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimized movement in fields, especially when foliage is wet, may be effective (Allen *et al.* 1998, Schwartz & Otto 2000). Copper-based bactericides protect foliage against bacterial diseases and secondary pathogen spread. Efficacy of chemical control, however, is limited (Allen *et al.* 1998) and resultant yield increases are minimal (Saettler 1989).

The most important factor of an integrated approach is use of resistant cultivars (Rands & Brotherton 1925). Resistance breeding is, however, a long-term goal and emphasis should be placed on the disease with the highest economical impact on the bean industry. Effective deployment of resistance requires knowledge on pathogen variation, susceptibility of cultivars and resistance available in germplasm. The present study was undertaken to investigate these aspects and to use this knowledge in a breeding programme, especially focusing on common bacterial blight.

REFERENCES

Allen, D.J., Buruchara, R.A. & Smithson, J.B. (1998) Diseases of common bean. In *The pathology of food and pasture legumes* (D.J. Allen & J.M. Lenné, eds) : 179-235. CAB International, Wallingford.

Coetzee, C. (2000) Droëboneproduksies, die verlede, die hede en 'n scenario vir 2001. *SA Droëbone/Dry beans* **16**: 2-3.

De León, L.F., Elías, L.G. & Bressani, R. (1992) Effect of salt solutions on the cooking time, nutritional and sensory characteristics of common bean. *Food Research International* **25**: 131-136.

Fourie, D. (2002) Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology* **150**: 220-226.

Gilbertson, R.L., Rand, R.E. & Hagedorn, D.J. (1990) Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. *Plant Disease* **74**: 322-327.

Jensen, J.H. & Livingston, E. (1944) Variation in symptoms produced by isolates of *Phytomonas medicaginis* var. *phaseolicola*. *Phytopathology* **34**: 471-480.

Paradez-López, O., Montes-Ribera, R., Gonzàlez Castaneda, J. & Arroyo-Figueroa, M.G. (1986) Comparison of selected food characteristics of three cultivars of bean *Phaseolus vulgaris*. *Journal of Food Technology* **21**: 487-494.

Rands, R.D. & Brotherton, W. (1925) Bean varietal tests for disease resistance. *Journal of Agricultural Research* **31**: 110-154.

Saettler, A.W. (1989) Common bacterial blight. In *Bean Production Problems in the Tropics 2nd ed* (H.F. Schwartz & M.A. Pastor-Corrales, eds) : 261-283. CIAT, Cali, Colombia.

Saettler, A.W. (1991) Diseases caused by bacteria. In *Compendium of bean diseases* (R. Hall, ed) : 29-32. APS-Press, St. Paul, Minnesota.

Schwartz, H.F. (1989) Halo blight. In *Bean Production problems in the Tropics* (H.F. Schwartz & M.A. Pastor-Coralles, eds) CIAT, Cali, Colombia.

Schwartz, H.F. & Otto, K.L. (2000) Enhanced bacterial disease management strategy. *Annual Report of the Bean Improvement Cooperative* **43**: 37-38.

Serfontein, J.J. (1994) Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. *Plant Pathology* **43**: 597-599.

Singh, S.P. (1999) Production and Utilization. In *Common Bean Improvement in the Twenty-First Century* (S.P. Singh, ed) : 1-24. Kluwer Academic Publishers, London.

Taylor, J.D., Phelps, K. & Dudley, C.L. (1979) Epidemiology and strategy for the control of halo blight of beans. *Annals of Applied Biology* **93**: 167-172.

Watson, D.R.W. (1980) Identification of bacterial brown spot of bean in New Zealand. *New Zealand Journal of Agricultural Research* **23**: 267-272.

Yoshii, K. (1980) Common and fuscous blights. In *Bean Production problems in the Tropics* (H.F. Schwartz & M.A. Pastor-Corralles, eds) : 157-172. CIAT, Cali, Colombia.

Figure 1. Common bacterial blight on dry bean leaves caused by *Xanthomonas axonopodis* pv. *phaseoli*

Figure 2. Common bacterial blight (*X. axonopodis* pv. *phaseoli)* on dry bean pods

Figure 3. Leaf symptoms of dry bean halo blight caused by *Pseudomonas savastanoi* pv. *phaseolicola*

Figure 4. Halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) on dry bean pods

Figure 5. Dwarfed bean plant systemically infected with halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*)

Figure 6. Bacterial brown spot (*Pseudomonas syringae* pv. *syringae*) symptoms on dry bean leaves

Figure 7. Pod symptoms of bacterial brown spot (*Pseudomonas syringae* pv. *syringae*)

CHAPTER 2

DISTRIBUTION AND SEVERITY OF BACTERIAL DISEASES ON DRY BEANS (*PHASEOLUS VULGARIS* L.) IN SOUTH AFRICA

ABSTRACT

Disease surveys were conducted during 1995/96 in seed production fields, and 1996/97 and 1997/98 in commercial dry bean producing areas to determine incidence, severity and spread of bacterial diseases in South Africa. Six-hundred-and-eighty-two seed production fields at 31 localities and 81 commercial fields at 24 localities were surveyed. Common bacterial blight occurred in 83% and 85% of localities in seed and commercial production areas, respectively. Halo blight was restricted to cooler production areas and occurred in only 10% of seed production fields and 37% of commercial fields surveyed. Bacterial brown spot was the most widespread bacterial disease occurring in 93% of seed production fields and 100% commercial fields. Although incidences of bacterial diseases were high, severity was generally low. The widespread distribution of bacterial diseases in both seed and commercial production areas questions the effectivity of disease-free seed as primary control method.

Fourie, D. (2002) Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology* **150**: 220-226.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) play an important role in crop production systems in Africa and are the second most important plant protein source after groundnuts (Technology Impact Report 1998). Mean production of 58 000 t on 56 000 ha has been recorded in South Africa for the past 10 years (Coetzee 2000). Beans are produced commercially in Mpumalanga (56%), Free State (28%), North West (7%), KwaZulu-Natal (5%) and Northern Cape (4%) provinces. The major areas for small-scale farmer bean production are Mpumalanga, Eastern Cape Province and KwaZulu-Natal. Seed types grown primarily are red speckled sugar (79%), small white (12%) and large white (*P. coccineus* - 4%) beans. Seed types of lesser importance (5%) include carioca, haricot and alubia beans (Coetzee 2000).

Bacterial diseases are a major constraint limiting South African dry bean production. Locally occurring bacterial diseases are common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*, [Xap] and its fuscans variant *X. axonopodis pv. phaseoli var. fuscans*, [Xapf]), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*, [Psp]) and bacterial brown spot (*P. syringae* pv. *syringae*, [Pss]). Incidence and severity of these diseases vary annually, being influenced by biological and climatic factors as well as management practices.

Common blight is a worldwide problem in bean production and may be highly destructive during extended periods of warm, humid weather, resulting in yield and seed quality losses (Saettler 1991). Common blight, and fuscous blight are often referred to as separate diseases (Boelema 1967). Xapf differs from Xap in that it produces a brown diffusible pigment in culture media. Although several reports exist that Xapf is more

virulent than Xap (Leakey 1973, Ekpo & Saettler 1976, Bozzano-Saguier & Rudolph, 1994, Opio *et al.* 1996), it has been indicated that the Xapf pigment is not associated with pathogenicity (Gilbertson *et al.* 1991, Tarigan & Rudolph 1996) and could be considered of lesser pathological importance (Schuster & Coyne 1975). The respective common blight bacteria produce identical symptoms on susceptible bean plants and will be discussed as a single disease.

Common blight is usually visible during the crop's reproductive stage. The disease has been reported in 19 of the 20 bean producing countries in Eastern and Southern Africa (Allen 1995) and is one of the five most important biotic constraints of dry bean production in sub-Saharan Africa (Gridley 1994). Although common blight is widely distributed, yield losses have not been well documented, but have been reported to vary between 22% and 45% (Wallen & Jackson 1975, Yoshii 1980).

Halo blight is distributed worldwide and is favoured by cool, wet weather early in the season. Yield losses of 43% have been obtained under experimental conditions. In Africa, serious crop losses have been observed in Lesotho, Rwanda and Zimbabwe (Allen *et al.* 1998).

Bacterial brown spot results in sporadic losses in moderate to hot production areas, especially where plants have been damaged by heavy rain or hail (Serfontein 1994). During 1992, disease incidence was 100% in plantings in Mpumalanga and yield reductions estimated at 55% (Serfontein 1994). Bacterial brown spot occurs throughout bean production areas worldwide and is a serious constraint in snap bean production in the United States of America (USA) (Schwartz 1980). Although widespread in Africa (Allen 1995), bacterial brown spot is considered a disease of minor importance.

Bacterial bean pathogens are seed-borne, this being the primary inoculum source (Allen *et al.* 1998). Planting of disease-free seed is therefore an important primary control method (Zaumeyer & Thomas 1957), and for this reason a disease free seed scheme was introduced in South Africa in 1980 (A.J. Liebenberg, Agricultural Research Council: personal communication). Disease-free seed is produced in Northern Province, northern parts of Mpumalanga and Northern Cape during winter when climatic conditions and rigid quarantine minimize risk of infestation by bacterial pathogens. Seed certification schemes are also successfully implemented in the USA (Copeland *et al.* 1975), Canada (Sutton & Wallen 1970) and Australia (Redden & Wong 1995).

Seed production fields are regularly inspected for presence of disease and fields with visible bacterial infection levels >8% are rejected, whereas fields with <8% infection are regarded acceptable for certified seed. Seed from fields showing no visible disease symptoms are laboratory tested and certified disease free after absence of specific pathogens has been confirmed (W. Havenga, Dry Bean Producers Organization: personal communication).

Despite implementation of the Seed Scheme, damaging epidemics still occur in commercial and seed production areas during wet seasons (D. Fourie: unpublished data). The aim of this survey was to determine incidence, severity and occurrence of bacterial diseases in commercial and seed production fields over three seasons (1995/96 in seed production fields and 1996/97 and 1997/98 in commercial fields). Information obtained from this survey should influence the use of integrated control strategies for effective control of bacterial diseases on dry beans in South Africa.

MATERIAL AND METHODS

Disease survey in seed production fields

Six hundred and eighty two seed production fields at 31 localities were surveyed from March to August (depending on planting date) during 1996 for visual bacterial disease symptoms. Fields were inspected at flowering and full pod set. Two hundred randomly selected plants per field were inspected for presence of typical symptoms. For the seed certification scheme severity was not considered, only percentage disease incidence.

Disease surveys in commercial fields

Bacterial disease surveys of commercial fields were conducted from February to March, during the 1996/97 and 1997/98 growing season, in commercial bean production areas to determine incidence, severity and spread. Eighty-one fields at 24 localities were surveyed prior to flowering, during flowering or at early-pod set, depending on planting-date. Incidence (percentage) of plants showing typical bacterial disease symptoms were assessed in five randomly selected groups of 20 consecutive plants (100 plants/field). Disease severity was evaluated for each plant on a 0-4 scale (0=no symptoms; 1=1-33% foliage affected; 2=34-66% foliage affected; 3=67-100% foliage affected; 4=dead plant) (Bejarano-Alcázar *et al.* 1996). Incidence and severity values were used to calculate a disease index (D_I), using the model: $D_I = (I \times S)/M$ where I=incidence of diseased plants (%), S=mean severity of foliar symptoms and M=maximum severity value (Bejarano-Alcázar *et al.* 1996).

Isolation of bacterial pathogens

Bacteria from seed production fields were isolated by soaking seed samples in a saline solution at 4°C for 24 hr. Serial dilutions were plated on King's B medium (King *et al.* 1954) and yeast-extract-dextrose-calcium-carbonate agar (YDC) (Schaad & Stall 1988) and incubated at 25°C.

Diseased leaves sampled from each commercial field surveyed were used to isolate bacteria. Leaves were rinsed under running tap water for 10 min, surface-sterilized for 3 min in 3,5% sodium hypochlorite and then rinsed twice in sterile water for 1 min each. Leaves were macerated in a droplet of sterile water and macerate streaked onto King's B and YDC agar. Plates were incubated at 25°C.

Identification of bacterial pathogens

Following 72 hr incubation, yellow pigmented colonies typical of *Xanthomonas* spp. were purified on YDC agar by a series of single colony transfers. Production of brown diffusible pigment on YDC differentiated Xapf from Xap isolates (Basu & Wallen 1967). Antiserum specific to Xap and Xapf, obtained from Adgen Agrifood Diagnostics, Auchincruive, Scotland, were used to confirm identity of isolates. Xap and Xapf isolates were inoculated with a multiple needle (Andrus, 1948) onto first trifoliate leaves of the cultivar Teebus to determine pathogenicity.

Fluorescent colonies typical of *Pseudomonas* spp. were selected under UV-light and purified on King's B medium after 48 hr incubation. Isolates were tested for oxidase (-) and levan production (+). Carbon source utilization of sucrose, mannitol, sorbitol and inositol were used to distinguish Pss from Psp isolates (Hildebrand *et al.* 1988).

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Agglutination of Psp and Pss antiserum confirmed identity of isolates. Seven- to 10-day-old seedlings of susceptible cultivar Canadian Wonder, were spray-inoculated with Psp isolates (Taylor *et al.* 1996) to confirm pathogenicity. Young attached pods of Teebus plants were inoculated with Pss isolates using the method of Cheng *et al.* (1989).

RESULTS

Distribution of bacterial diseases in seed production and commercial fields

Occurrence of bacterial diseases in dry bean seed production areas and commercial fields is indicated in Tables 1 and 2, respectively. Weather data from surveyed localities are shown in Table 3. Common bacterial blight (Xap, Xapf) occurred in 83% of seed production areas and in 79% commercial fields. Incidences and severities in commercial fields were low, except at Nigel where incidence was 85%. According to laboratory tests, common blight is more widely distributed than indicated in visual field surveys. Although disease symptoms were not noted at Carletonville, Clocolan, Grootpan and Vryheid, the common blight pathogen was isolated from diseased leaves with typical brown spot lesions collected in these areas (Table 2). Petrus Steyn (Free State) was the only locality from which the common blight pathogen was not isolated (Table 2). The fuscans variant, Xapf, was more widespread than Xap in both seed production and commercial fields.

Halo blight (Psp) occurred in only three seed production localities (10%) in the Northern Cape, Northern Province and North West Province. In commercial fields, halo

blight was restricted to cooler production areas and occurred at 37% of localities. Incidence and severity were low with disease indexes ranging from 0,5-24,5.

Bacterial brown spot (Pss) was the most widespread bacterial disease and occurred in 93% seed production and 100% commercial fields, respectively. Although disease incidences were high (up to 100%) severities were generally low (1-2).

DISCUSSION

Planting of disease-free seed is the primary control measure for bacterial diseases of dry beans in South Africa. In addition, copper based bactericides are used to protect foliage against bacterial infestation and secondary spread. However, efficacy of chemical control of bacterial diseases is limited and resultant yield increases have been reported to be minimal (Saettler 1989).

The widespread occurrence of bacterial diseases in seed production areas impacts strongly on the use of disease-free seed as sole local control strategy. Bacterial pathogen infections in seed production areas significantly limited disease-free seed availability during 1996/97. Fifty-one seed production fields (7,5%) were rejected due to infections exceeding 8%. Although visual symptoms were not always observed, pathogens detected during laboratory seed testing resulted in seed rejection. The majority of seed produced (61,8% of fields surveyed) was classified as certified seed with infection levels less than 8%. Only 36 fields surveyed (5,3%) were certified as disease-free.

Local classification of seed as 'certified' and 'certified disease-free', is confusing. Infection levels of 8%, permitted for certified seed, could have serious implications on

commercial production, when considering that 0,5% seed infestation can cause serious outbreaks of bacterial diseases (Sutton & Wallen 1970). On the other hand, zero tolerance in certified disease free seed is impossible to achieve as this would require testing of the entire stock (Taylor *et al.* 1979). Sampling techniques are, therefore, of utmost importance and local seed certification should aim at levels as near to zero percent infestation as possible.

Certification standards in the USA permit 0,005% infected plants during field inspection and no infected seed upon laboratory examination. Infected seed production fields in Idaho are immediately ploughed in and destroyed (Copeland *et al.* 1975). Production under these conditions provides a continuous supply of certified seed that contributes significantly towards disease management of bacterial blight (Sutton & Wallen 1970, Copeland *et al.* 1975).

Isolation of production fields is insufficient in South Africa and problems are encountered with seed production fields which are sometimes in close proximity of commercial fields. New seed production areas need to be obtained. Neighbouring countries such as Zimbabwe and Mozambique could be considered. Although rainfall in seed production areas is low, occurrence of common blight and bacterial brown spot could be favoured by prevailing temperatures. Dissemination of bacterial pathogens between bean fields has been shown to occur by aerosols generated during dry, sunny, windy weather (Hirano *et al.* 1995).

The high incidence of bacterial brown spot and common blight in seed production fields during 1996 was probably responsible for its widespread occurrence in commercial fields during 1996/97 and 1997/98. Although common blight was observed in 79% commercial fields, the causal pathogen was isolated from 96% of localities surveyed.

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Common blight is potentially more devastating as no resistant cultivars are available in South Africa.

Incidence and severity of halo blight were low in both seed production and commercial fields. The pathogen was restricted to higher altitudes and favoured by low night temperatures. Although climatic conditions in seed production areas are generally unfavourable for halo blight development, low night temperatures could have contributed to occurrence of disease in Prieska, Messina and Tosca. *P. coccineus* (white large kidney beans), highly susceptible to halo blight, is not included in the seed certification scheme and could have contributed to incidence of disease in commercial fields in Ermelo and Bergville. *P. coccineus* production is restricted to cooler areas that also favour development of halo blight. Although halo blight incidence in commercial and seed production fields was low, this disease has caused considerable yield losses particularly where farmers grow their own seed for two or more years (D. Fourie: unpublished data).

A strong association has been shown to exist between rainfall and onset of epidemic growth in pathogen populations (Hirano *et al.* 1995). These authors reported that *P. syringae* pv. *syringae* populations increased almost 100-fold from 34 to 35 days after planting (DAP) after 26 mm rainfall on 34 DAP. It can be concluded that higher than average rainfall in commercial production areas could have contributed to the occurrence and spread of bacterial diseases.

From information gained from the survey it can be concluded that measures used to control bacterial diseases in South Africa are insufficient. The widespread occurrence of bacterial diseases in South Africa suggest that the use of disease-free seed alone does not guarantee freedom of bacterial diseases. The occurrence of bacterial

diseases, in particular halo blight, has however, decreased significantly since implementation of the disease-free seed scheme. Improvement of local cultivar resistance is important for long term control of bacterial diseases. An integrated disease management system which includes resistant cultivars, disease free seed (produced in more suitable areas), agricultural practices and preventative spraying with copper-based bactericides should reduce occurrence of bacterial pathogens.

REFERENCES

Allen, D.J. (1995) An annotated list of diseases, pathogens and associated fungi of the common bean (*Phaseolus vulgaris*) in Eastern and Southern Africa. *Phytopathological Papers* **34**. CAB International/Centro Internacional de Agricultura Tropical, Cali, Colombia.

Allen, D.J., Buruchara, R.A. & Smithson, J.B. (1998) Diseases of common bean. In *The pathology of food and pasture legumes* (D.J. Allen & J.M. Lenné, eds) : 179-235. CAB International, Wallingford.

Andrus, C.F. (1948) A method of testing beans for resistance to bacterial blights. *Phytopathology* **38**: 757-759.

Basu, P.K. & Wallen, V.R. (1967) Factors affecting virulence and pigment production of *Xanthomonas phaseoli* var. *fuscans*. *Canadian Journal of Botany* **45**: 2367-2374.

Bejarano-Alcázar, J., Blanco-López, M.A, Melero-Yara, J.M. & Jiménez-Díaz, R.M. (1996) Etiology, importance, and distribution of Verticillium wilt of cotton in southern Spain. *Plant Disease* **80**: 1233-1238.

Boelema, B.H. (1967) Fuscous blight of beans in South Africa. *South African Journal of*

Agricultural Science **10**: 1059-1063.

Bozzano-Saguier, G. & Rudolph, K. (1994) Differential reactions of bush bean cultivars towards common and fuscous blight (*Xanthomonas campestris* pv. *phaseoli* and *X. c. phaseoli* var. *fuscans*). *Annual Report of the Bean Improvement Cooperative* **37**: 227-228.

Cheng, G.Y., Legard, D.E., Hunter, J.E. & Burr, T.J. (1989) Modified bean pod assay to detect strains of *Pseudomonas syringae* pv. *syringae* that cause bacterial brown spot of snap bean. *Plant Disease* **73**: 419-423.

Coetzee, C. (2000) Droëboneproduksies, die verlede, die hede en 'n scenario vir 2001. *SA Droëbone/Dry beans* **16**: 2-3.

Copeland, L.O., Adams, M.W. & Bell, D.C. (1975) An improved seed programme for maintaining disease-free seed of field beans (*Phaseolus vulgaris*). *Seed Science Technology* **3**: 719-724.

Ekpo, E.J.A. & Saettler, A.W. (1976) Pathogenic variation in *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans*. *Plant Disease Reporter* **60**: 80-83.

Gilbertson, R.L., Otoya, M.M., Pastor-Corrales, M.A. & Maxwell, D.P. (1991) Genetic diversity in common blight bacteria is revealed by cloned repetitive DNA sequences. *Annual Report of the Bean Improvement Cooperative* **34**: 37-38.

Gridley, H.E. (1994) Bean production constraints in Africa with special reference to breeding for resistance to bean common mosaic virus in Uganda. In *Proceedings of a Pan-African working group meeting on bacterial and virus diseases of common bean. CIAT African Workshop Series* (D.J. Allen & R.A. Buruchara, eds) **34**: 33-39. Kampala, Uganda.

Hildebrand, D.C., Schroth, M.C. & Sands, D.C. (1988) Pseudomonas. In *Laboratory*

i16378131
b15820439

Guide for Identification of Plant Pathogenic Bacteria. 2nd ed (N.W. Schaad, ed) : 60-81. The American Phytopathological Society, St. Paul, MN, USA.

Hirano, S.S., Rouse, D.I., Clayton, M.& Upper, C.D. (1995) *Pseudomonas syringae* pv. *syringae* and bacterial brown spot of snap bean: a study of epiphytic phytopathogenic bacteria and associated disease. *Plant Disease* **79**: 1085-1093.

King, E.O., Ward, M.K. & Raney, D.E. (1954) Two simple media for demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**: 301-307.

Leakey, C.L.A. (1973) A note on *Xanthomonas* blight of beans (*Phaseolus vulgaris* (L.) savi) and prospects for its control by breeding for tolerance. *Euphytica* **22:** 132-140.

Opio, A.F., Allen, D.J. & Teri, J.M. (1996) Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathology* **45**: 1126-1133.

Redden, R.J. & Wong, W.C. (1995) Detection and elimination of common blight from common bean seed. *Annual Report of the Bean Improvement Cooperative* **38**: 164-165.

Saettler, A.W. (1989) Common bacterial blight. In *Bean Production Problems in the Tropics 2nd ed* (H.F. Schwartz & M.A. Pastor-Corrales, eds) : 261-283. CIAT, Cali, Colombia.

Saettler, A.W. (1991) Diseases caused by bacteria. In *Compendium of bean diseases* (R. Hall, ed) : 29-32. APS-Press, St. Paul, Minnesota.

Schaad, N.W. & Stall R.E. (1988) Xanthomonas. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria 2nd ed* (N.W. Schaad, ed) : 81-93. The American

Phytopathological Society, St. Paul, MN, USA.

Schwartz, H.F. (1980) Bacterial diseases. In *Bean production problems: disease, insect, soil and climatic constraints of Phaseolus vulgaris* (H.F Schwartz & G.E. Gálvez, eds) : 55-187. CIAT, Cali, Colombia.

Schuster, M.L. & Coyne, D.P. (1975) Genetic variation in bean bacterial pathogens. *Euphytica* **24**: 143-147.

Serfontein, J.J. (1994) Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. *Plant Pathology* **43**: 597-599.

Sutton, M.D. & Wallen, V.R. (1970) Epidemiological and ecological relations of *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* on beans in southwestern Ontario, 1961-1968. *Canadian Journal of Botany* **48**: 1329-1334.

Tarigan, J.R. & Rudolph, K. (1996) Investigations on the resistance of bean genotypes to *Xanthomonas campestris* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* and on the differentiation of bacterial strains. *Annual Report of the Bean Improvement Cooperative* **39**: 284-285.

Taylor, J.D., Phelps, K. & Dudley, C.L. (1979) Epidemiology and strategy for the control of halo blight of beans. *Annals of Applied Biology* **93**: 167-172.

Taylor, J.D., Teverson D.M., Allen, D.J. & Pastor-Corrales, M.A. (1996) Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. *Plant Pathology* **45**: 469-478.

Technology Impact Report. (1998) ARC-Grain Crops Institute, Oil and Protein Seed Centre, Potchefstroom.

Wallen, V.R. & Jackson, H.R. (1975) Model for yield loss determination of bacterial blight of field beans utilizing aerial infrared photography combined with field plot studies.

Phytopathology **65**: 942-948.

Yoshii, K. (1980) Common and fuscous blights. In *Bean Production problems in the Tropics* (H.F. Schwartz & M.A. Pastor-Coralles, eds) : 157-172. CIAT, Cali, Colombia.

Zaumeyer, W.J. & Thomas, H.R. (1957) A monographic study of bean diseases and methods for their control. *United States Department of Agriculture Technical Bulletin* **868**: 74-84.

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Table 1. Occurrence of bacterial diseases in seed production areas of South Africa during 1996

Locality		Fields surveyed (Ha)	Dry bean cultivars	Bacteria identified in seed samples	R	C	DF
Northern Cape:							
	Hopetown	50 (172)	Helderberg, Kranskop, Donkerberg	Pss, Xapf, Xap	12	20	0
	Prieska	161 (565)	Teebus, Kranskop, Leeukop, Katberg	Pss, Xapf, Xap, Psp	7	135	6
	Kimberley	15 (59)	Katberg, Teebus, Kranskop	Pss, Xapf	0	3	0
	Douglas	96 (399)	Teebus, Kranskop, Katberg, Donkerberg	Pss, Xapf, Xap	2	60	2
	Petrusville	6 (26)	Kranskop	Pss, Xapf	0	6	0
	Perdeberg	9 (32)	Kranskop, Leeukop, Donkerberg	Pss, Xapf	0	9	0
	Modderrivier	25 (92)	Kranskop, Leeukop	Pss, Xapf	0	15	0
	Landanna	7 (20)	Kranskop	Pss	7	0	0
	Hadeson park	26 (93)	Teebus, Katberg	Pss, Xapf	3	7	7
	Koffiefontein	2 (9)	Kranskop	Pss, Xapf	0	1	0
	Jacobsdal	24 (136)	Kranskop, Leeukop	Pss, Xapf	0	10	0
Northern Province:							
	Tzaneen	16 (75)	Helderberg	Pss, Xapf, Xap	0	9	7
	Hoedspruit	12 (136)	Kranskop	Pss, Xapf, Xap	1	9	2
	Dendron	27 (198)	Kranskop, Leeukop, Donkerberg, Katberg	Pss	5	10	1
	Pietersburg	14 (120)	Teebus, Kranskop	Pss, Xapf	4	6	0
	Vivo	23 (107)	Leeukop, Kranskop	Pss, Xapf, Xap	0	8	0
	Vaalwater	6 (50)	Leeukop	Pss, Xapf, Xap	0	2	0
	Messina	18 (108)	Helderberg, Kranskop	Pss, Psp	0	12	5
Mpumalanga:							
	Lydenburg	35 (337.5)	Kranskop, Bonus, Stormberg	Pss, Xapf, Xap	5	26	4
	Origstad	16 (162.5)	Kranskop, Bonus, Stormberg	Pss, Xap	0	1	0
	Badplaas	8 (181.9)	Kranskop, Stormberg	Pss, Xapf, Xap	0	3	0
	Nelspruit	8 (45.5)	Kranskop	Pss, Xapf, Xap	4	3	0
	Barberton	1 (14.1)	Kranskop	Pss	1	0	0
	Witrivier	10 (55)	Kranskop	Pss, Xapf, Xap	0	10	0
	Burgersfort	4 (37.2)	Stormberg	Xapf, Xap	0	4	0
Free State:							
	Petrusburg	1 (26)	Donkerberg	Pss, Xapf	0	1	0
	Luckhoff	2 (6)	Kranskop	Pss, Xapf, Xap	0	1	0
Northwest:							
	Vryburg	36 (135)	Kranskop	Pss, Xapf, Xap	0	32	2
	Tosca	23 (85)	Kranskop	Pss, Xapf, Xap, Psp	0	18	0
TOTAL:		**681 (3482.7)**			**51**	**421**	**36**

R = number of fields rejected, C = number of fields certified (< 8.0% disease), DF = number fields classified as disease free (0% diseases); Pss = *P. syringae* pv. *syringae*, Psp = *P. savastanoi* pv. *phaseolicola*, Xap = *X. axonopodis* pv. *phaseoli*, Xapf = *X. axonopodis* pv. *phaseoli* var. *fuscans*

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Table 2. Incidence and severity of bacterial diseases in the main commercial dry bean production areas of South Africa during 1996/97 and 1997/98

Locality	Fields surveyed (No of diseased fields)	Cultivars	Bacteria isolated from diseased leaves	Common Bacterial Blight			Halo Blight			Bacterial Brown Spot		
				I (%)	*S* (1-4)	D_i (%)	*I* (%)	*S* (1-4)	D_i (%)	*I* (%)	*S* (1-4)	D_i (%)
Mpumalanga:												
Delmas	7 (4)	Teebus, Kranskop, Pan 148	Pss, Xapf, Xap	24.0	1.18	7.1	0	-	-	27.5	1.04	7.2
Ermelo	9 (9)	Teebus, Leeukop, Mkuzi, *P. coccineus*	Pss, Xapf, Xap, Psp	38.2	1.06	10.1	15.8	1.28	10.8	17.7	1.00	4.4
Ogies	2 (2)	Cerrillos, Pan 148	Pss, Xapf, Xap	21.0	1.00	5.3	0	-	-	95.0	1.27	30.5
Gauteng:												
Nigel	2 (2)	Kranskop, Cerrillos, Sabie	Pss, Xapf, Xap	85.0	1.16	24.5	0	-	-	3.0	1.00	0.75
Carletonville	1 (1)	-	Pss, Xapf, Xap	0	-	-	0	-	-	100.0	1.70	43.0
Free State:												
Bethlehem	11 (8)	Donkerberg, Teebus, Sabie, Bonus, Stormberg, Kranskop	Pss, Xapf, Xap, Psp	8.6	1.02	2.3	13.9	1.08	4.2	32.6	1.03	8.5
Clarens	5 (5)	Leeukop, Stormberg, Pan 148	Pss, Xapf, Xap, Psp	25.8	1.00	6.5	16.2	1.00	4.1	58.8	1.32	23.4
Harrismith	3 (3)	Kranskop	Pss, Xapf, Xap, Psp	26.3	1.05	7.0	12.3	1.00	3.1	99.3	1.57	39.1
Reitz	2 (2)	Kranskop	Pss, Xapf, Psp	6.5	1.00	1.63	2.0	1.00	0.5	90.5	1.29	28.7
Fouriesburg	1 (1)	Stormberg	Pss, Xapf, Xap, Psp	1.0	1.00	0.25	44.0	1.18	13.0	4.0	1.00	1.0
Petrus Steyn	1 (1)	Kranskop	Pss	0	-	-	0	-	-	90.0	1.00	22.5
Warden	1 (1)	-	Pss, Xapf	11.0	1.00	2.8	0	-	-	94.0	1.00	23.5
Clocolan	4 (4)	Kranskop, Pan 148	Pss, Xapf, Xap, Psp	0	-	-	60.8	1.45	24.6	25.0	2.03	0.5
North West:												
Syferbult	7 (6)	Pan 148, Kranskop, Teebus	Pss, Xapf, Xap	16.9	1.46	8.0	0	-	-	46.4	1.25	16.8
Koster	3 (2)	-	Pss, Xapf, Psp	3.0	1.25	0.9	3.3	1.00	0.8	33.3	2.40	20.0
Derby	1 (1)	-	Pss, Xapf, Xap	17.0	1.00	4.25	0	-	-	17.0	1.00	4.3
Grootpan	3 (3)	Kranskop	Pss, Xapf, Xap, Psp	0	-	-	13.7	1.5	5.1	96.3	1.10	26.6
Coligny	3 (3)	Helderberg	Pss, Xapf, Xap	17.3	1.00	4.3	0	-	-	30.0	1.01	7.6
KwaZulu/Natal:												
Vryheid	5 (4)	Helderberg, Wartburg, Enseleni, Kranskop, Pan 148	Pss, Xapf	0	-	-	0	-	-	50.0	1.00	12.5
Greytown	3 (3)	Mkuzi	Pss, Xapf, Xap	28.6	1.00	7.8	0	-	-	48.7	1.01	12.3
Middelrus	3 (3)	Kranskop	Pss, Xapf, Psp	2.7	1.00	0.7	3.3	1.20	1.0	80.0	1.09	22.2
Newcastle	1 (1)	Sabie	Pss, Xapf	14.0	1.00	3.5	0	-	-	28.0	1.00	7.0
Winterton	1 (1)	Kranskop	Pss, Xapf, Xap	10.0	1.00	2.5	0	-	-	100.0	1.26	31.5
Bergville	2 (2)	Limpopo, *P. coccineus*	Pss, Xapf, Xap, Psp	17.0	1.00	4.3	10.0	1.00	2.5	33.0	1.00	8.3
TOTAL:	**81 (72)**			**19.6**	**1.01**	**5.46**	**17.8**	**1.15**	**6.3**	**54.2**	**1.22**	**16.8**

I = Incidence of plants with foliar symptoms; *S* = Disease severity; D_i = Disease intensity index

Table 3. Weather data from dry bean localities surveyed

Locality	Altitude	[a]Rainfall (mm)	[b]Min T	[c]Max T
Northern Cape:				
Prieska †	-	1.4	4.9	18.5
Kimberley †	1140	1.2	1.3	20.0
Douglas	1082	-	-	-
Petrusville	1143	14.7	6.6	21.5
Perdeberg	1173	16.4	-	-
Koffiefontein	-	19.2	-	-
Jacobsdal	-	29.9	-	-
Northern Province:				
Tzaneen	884	67.7	12.7	22.1
Hoedspruit	550	18.0	12.9	25.4
Dendron	1067	23.6	-	-
Pietersburg	1250	22.6	6.1	21.5
Vivo	1067	23.6	-	-
Vaalwater	1215	15.9	7.6	23.1
Messina	522	-	-	-
Mpumalanga:				
Lydenburg	1644	32.5	7.5	20.9
Orighstad	1525	15.7	5.9	20.3
Badplaas	1100	43.2	9.3	22.3
Nelspruit	660	52.7	10.6	24.0
Barberton	1300	73.9	9.6	19.8
Witrivier	676	44.8	13.3	24.7
Burgersfort	915	-	-	-
Delmas	1623	135.8	13.7	25.5
Ermelo	1765	52.7	13.3	24.3
Ogies	1550	69.3	-	-
Free State:				
Petrusburg	1219	19.8	4.3	21.3
Bethlehem	1631	11.8	13.2	24.9
Clarens	-	-	-	-
Harrismith	1718	97.6	13.6	25.2
Reitz	1615	116.9	13.7	25.9

† Data available for August only; [a] mean rainfall from March to August in seed production fields, February to March in commercial fields;
[b] mean minimum temperatures from March to August in seed production fields, February to March in commercial fields;
[c] mean maximum temperatures from March to August in seed production fields, February to March in commercial fields

CHAPTER 3

CHARACTERIZATION OF HALO BLIGHT RACES ON DRY BEANS IN SOUTH AFRICA

ABSTRACT

Isolates of the halo blight pathogen *Pseudomonas savastanoi* pv. *phaseolicola* were collected in the bean producing areas in South Africa from 1991 to 1996. Of the 1128 isolates, 967 were identified as *P. savastanoi* pv. *phaseolicola*. The majority of these isolates were obtained from a wide range of *Phaseolus vulgaris* cultivars and the rest from *P. coccineus* and *P. lunatus*. Two hundred and fifty five isolates, representative of all the localities and cultivars sampled, were categorized into different races according to their reaction on a set of differential cultivars. Seven races (1, 2, 4, 6, 7, 8 and 9) were identified with race 8 the most prevalent. Races 1, 2, 6 and 8 were widely distributed through the whole production area, while races 4, 7 and 9 were restricted to one or two localities.

Fourie, D. (1998) Characterization of halo blight races on dry beans in South Africa. *Plant Disease* **82**: 307-310.

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INTRODUCTION

Halo blight, caused by *Pseudomonas savastanoi* pv. *phaseolicola* (Burkh.) Gardan *et al.*, is an important seed-borne disease of dry beans (*Phaseolus vulgaris* L.) (Buruchara 1983, Beebe & Pastor-Corrales 1991). The disease is a major constraint of dry bean production in South Africa, especially in the moderate to cooler areas of the country. The extent of yield losses has not yet been estimated, but the disease occurs on all commercial cultivars and in many parts of the dry bean production areas.

Several races of *P. savastanoi* pv. *phaseolicola* have been reported worldwide. Races 1 and 2 have originally been described in the United States by Patel & Walker (1965) on their reaction to the cultivar Red Mexican U13. These races have since been reported from several other countries (Wharton 1967, Taylor 1970, Hale & Taylor 1973, Buruchara 1983). A third race from Africa was identified on the basis of its reaction to cv. Tendergreen (Mabagala & Saettler 1992). Recently, Taylor *et al.* (1996) extended the range of differentials to eight cultivars and lines and accordingly identified nine races of *P. savastanoi* pv. *phaseolicola*. Races 1 and 2 have previously been reported in South Africa (Boelema 1984, Edington 1990) on the basis of their reaction to Red Mexican U13. The aim of this study was to identify local races by using the extended range of cultivars, and to determine their geographic distribution.

MATERIALS AND METHODS

Sampling and isolation of bacteria

Leaves and pods of dry beans with halo blight symptoms were collected from the major bean producing areas in South Africa from 1991 to 1996. Samples were taken from various cultivars of *P. vulgaris*, *P. coccineus* L. (large white kidney beans) and *P. lunatus L.* (lima beans) and were collected from 255 disease occurrences. Prior to isolation, pods and leaves were rinsed under running tap water for 10 min and then surface sterilized by soaking material for 3 min in 3.5% sodium hypochlorite and rinsing it twice in sterile water for 1 min each. Bacteria were isolated using the method of Hildebrand *et al.* (1988) and streaked onto King's B medium (King *et al.* 1954). After incubation for 48 hr, fluorescent colonies were selected under UV-light and purified on King's B medium by a series of single colony transfers. Non-fluorescent colonies reminiscent of *Pseudomonas* in culture were additionally selected for further identification. All isolates are maintained at -72°C (Sleesman & Leben 1978) at the ARC-Grain Crops Institute, South Africa.

Identification of isolates

Carbon source utilization of mannitol, sorbitol and inositol, oxidase test and levan production (Hildebrand *et al.* 1988) as well as symptomology on leaves of cv. Canadian Wonder (universal susceptible cultivar) were used to confirm the identity of isolates. Antiserum specific to *P. savastanoi* pv. *phaseolicola* was provided by Dr. Nigel Lyons, of the Horticultural Research International (HRI), Wellesborne, England, during the latter part of the study and was additionally used to confirm the identity of isolates using the method of Taylor (1970).

Race identification

Two hundred and fifty five isolates representative of all the localities and cultivars sampled, were randomly selected for identification of races (Table 1). Seven reference cultures (1302A, 1299A, 2709A, 882, 1281A, 1449B, 2656A) of *P. savastanoi* pv. *phaseolicola* (Teverson 1991), obtained from Dr. Nigel Lyons were included for comparison to the races in South Africa. The isolates were kept on King's B agar slants at 4°C for the duration of the study.

Races were identified according to their reaction on a set of differential dry bean cultivars and lines (Teverson 1991, Taylor *et al.* 1996). Seeds of the differential set were planted in 8-cm-diameter plastic pots in sterile soil and maintained in a greenhouse at a 27°C /19°C day/night cycle of 12 hr each. Seeds from cv. 1072 were treated with 98% sulphuric acid for 30 min and kept overnight in moist paper rolls to germinate. For each isolate, three seeds were planted per pot and three pots used per differential. Pots were randomized prior to inoculation.

Inoculum was prepared by suspending a 24- to 48-hr-old culture in sterile distilled water and adjusting it turbimetrically to contain approximately 10^8 CFU/ml. Seven- to 10-day-old seedlings with fully expanded primary leaves were used for inoculation. Plants were inoculated with a DeVilbiss atomiser by spraying the bacterial suspension in two small areas (0.5 mm diameter) either side of the midrib onto the abaxial surface of the leaves, thereby forcing the bacteria into the leaf tissue (Zaiter & Coyne 1984, Teverson 1991, Taylor *et al.* 1996). The whole leaf area was then sprayed with the bacterial suspension until completely wet. Control plants were inoculated with sterile distilled water. Inoculated plants were kept in a humidity chamber (19°C±1°C,

RH=100%) for 48 hr before being transferred to a greenhouse equipped with a humidifier (18°C night/25°C day, RH=70%). Plants were rated for infection 10 days after inoculation on a 1 to 5 scale (Teverson 1991, Taylor *et al.* 1996) with 1 being highly resistant and 5 being highly susceptible.

Leaf vs pod inoculation

Twenty seven of the isolates selected for race identification were inoculated onto pods to compare whether pods and leaves react similarly to a specific race. Bacterial suspensions (10^8 CFU/ml) were inoculated onto young attached pods of the differential set with a hyperdemic needle using the modified method of Cheng *et al.* (1989). Sterile distilled water was used for control inoculations. Inoculated plants were maintained in a glasshouse (19°C night/27°C day±12 hr day length). Pods were rated for lesion development 10 days after inoculation.

RESULTS

Identification of isolates

A total of 1128 isolates were examined during the study. Nine hundred and sixty seven of the isolates were identified as *P. savastanoi* pv. *phaseolicola*. These isolates tested positive for levan, negative for the oxidase test and did not utilize mannitol, sorbitol and inositol as sole carbon sources. Water-soaked lesions developed when they were inoculated onto cv. Canadian Wonder. Systemic chlorosis as a result of toxin

translocation was also noted. Agglutination was observed with isolates tested with the antiserum specific to *P. savastanoi* pv. *phaseolicola*. A small percentage (2%) of the identified isolates were unable to produce fluorescent pigment on King's B medium and two isolates produced a brown diffusible pigment as described by Mabagala & Saettler (1992) and Taylor *et al*. (1996).

Race identification

Seven races of *P. savastanoi* pv. *phaseolicola* were identified in South Africa (Fig. 1). Of these, race 8 was the most prevalent (46.3%), while races 1, 2 and 6 constituted 27%, 6.3% and 18.6% of the isolates, respectively. Only four isolates of races 7 and 9 and one isolate of race 4 were found. Races 3 and 5 have not been identified from South African isolates of *P. savastanoi* pv. *phaseolicola*. The two isolates which produced a brown diffusible pigment on King's B belonged to race 1 and 6.

Race 8 was widely distributed and occurred in most of the localities sampled (Table 1). Race 1 was initially (during survey of 1991 to 1993) found only in the Mpumalanga Highveld where large white kidney beans are cultivated, but has since spread to new areas. Race 7 was confined to KwaZulu-Natal where it had been isolated from two localities (Cedara and Greytown) and race 9 was only found in KwaZulu-Natal and Mpumalanga. Although the occurrence of races 2 and 6 was low, these races were widespread throughout the production areas (Table 1).

The races identified were isolated from a wide variety of *P. vulgaris* and some *P. coccineus* and *P. lunatus* cultivars (Table 2). All seven races were identified from large seeded *P. vulgaris* cultivars. Five of the seven races were found on Wartburg, four

races on Bonus, SSB 10, 30 and 40 and green beans, while Stormberg, Umlazi and breeding trials hosted three races each. The rest of the large seeded cultivars were associated with one race only. Four races were identified from small seeded *P. vulgaris* cultivars, three from *P. coccineus* and one race from *P. lunatus* (Table 2). All three races of *P.coccineus* (races 1, 6 and 8) were found on cv. SSN1, while race 6 was isolated from SSN1, Bomba and Egyptian Great. Only one isolate, belonging to race 2, was found on *P. lunatus*.

Isolates from race 8 were identified from the majority of cultivars, while isolates belonging to races 1, 2 and 6 were also identified on a wide range of cultivars (Table 2). Race 4 was only found on imported kidney beans, race 7 on cv. Drakensberg and race 9 on cv. Umtata. Eighteen isolates consisting of races 1, 2, 6, 8 and 9 were collected from cultivars of which the names are unknown.

Leaf vs pod inoculation

Seventeen of the 27 isolates inoculated onto pods of the differential set gave similar race identifications as when inoculated onto primary leaves. These included isolates that belong to races 1, 2, 6, 7 and 8. Eight isolates were identified as race 8 when inoculated onto leaves but appeared to be race 6 when inoculated onto pods based on their reaction to cultivar A43. These isolates produced only a trace of water-soaking at the inoculation point on leaves of cv. A43 and were rated 2 on the infection rating scale, but produced water-soaked lesions on pods. Two isolates identified as race 1 on leaves also showed to be race 6 when inoculated onto pods. Only a trace of water-soaking (infection rating=2) was also observed on inoculated leaves of cvs. Red

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Mexican and Guatemala 196-B, but water-soaked lesions were clearly visible on pods.

DISCUSSION

Seven races of *P. savastanoi* pv. *phaseolicola* occurring on dry beans in South Africa were identified in this study. Previously only races 1 and 2 have been reported from the country (Edington 1990, Boelema 1994). The increased number of races occurring locally could be contributed either to the introduction of new races into South Africa, or the subdivision of the three previously described races into nine different races by using the extended range of differentials (Teverson 1991, Taylor *et al.* 1996). Also, the current study includes more isolates from more cultivars and a larger geographical area than those reported by Boelema (1984) and Edington (1990).

Race 8 dominated the South African population of *P. savastanoi* pv. *phaseolicola*. This is consistent with the results of Taylor *et al.* (1996) who found race 8 mainly in Lesotho and Southern Africa. It, therefore, appears that this race might have originated from this region. Another possible reason for the extensive occurrence of race 8 in South Africa is that the majority of cultivars planted locally are susceptible to it. Races 1, 2 and 6 also appear to be well established in South Africa, as each of them occurs on a variety of cultivars and in a number of localities. Three races (races 4, 7 and 9) were restricted to one or two localities, and it is likely that they have only recently been introduced. This hypothesis is supported by the fact that the only isolate belonging to race 4 was found on imported seed in a greenhouse trial, and does not occur in dry bean fields in South Africa. *P. savastanoi* pv. *phaseolicola* can be introduced into new areas on infected seed, and breeding programmes or planting of foreign seed can easily

result in the introduction and spread of new races in the country (Mabagala & Saettler 1992).

Various dry bean cultivars planted in South Africa were infected with halo blight in the field. This is of particular concern since the disease can be damaging. One means of controlling halo blight is by the introduction of a Disease Free Seed Scheme in South Africa. The exclusion from the Scheme of large white kidney beans, which is highly susceptible to halo blight, have probably resulted in the spread of race 1 in the country. Race 1 was initially found on large white kidney beans only, but has since been isolated from a number of *P. vulgaris* cultivars (D. Fourie, unpublished data). The planting of a large number of foreign cultivars during the past five years in the South African production areas could also have contributed to the introduction of new races.

Breeding for resistance provides the most effective means of control of halo blight (Beebe & Pastor-Corrales 1991, Mabagala & Saettler 1992). This study showed that a large number of cultivars planted locally are susceptible to *P. savastanoi* pv. *phaseolicola*. It is important that local cultivars and germplasm should be screened for resistance, and identified resistance be introduced into local cultivars. Seven races are present in South Africa and the possibility that new races could be introduced into the country exists. In order to breed for resistance, race non-specific resistance should be incorporated into local cultivars. Edmund and Wisc. HBR 72 are known to have race non-specific resistance and can be considered.

The inconsistent reactions of some isolates of *P. savastanoi* pv. *phaseolicola*, belonging to race 1 and 8, on leaves and pods of differentials A43, Red Mexican U13 and Guatemala 196-B could indicate that different genes are controlling pod and leaf resistance. Similar reactions have been reported by Hale & Taylor (1973). This

phenomena should be further investigated and molecular techniques could assist in confirming the race identification in isolates where indiscrepencies occur.

P. savastanoi pv. *phaseolicola* isolates which produce a brown diffusible pigment have been reported by several authors (Teverson 1991, Mabagala & Saettler 1992, Taylor *et al.* 1996). A similar pigment is often produced by isolates of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Saettler 1991). Two pigment producing bacteria, representing race 1 and race 6, were isolated from material collected from Leslie, situated in the cooler production areas of South Africa. The isolates reported by Mabagala & Saettler (1992) were identified as race 2. It seems as if the pigment production is not limited to a specific race, but its function is still unknown.

REFERENCES

Beebe, S.E. & Pastor-Corrales, M.A. (1991) Breeding for disease resistance. In *Common Beans, Research for Crop Improvement* (A. Van Schoonhoven & O. Voysest, eds) : 561-610. CAB International, Wallingford.

Boelema, B.H. (1984) Infectivity titrations with race 2 of *Pseudomonas syringae* pv. *phaseolicola* in green beans (*Phaseolus vulgaris*). *Phytophylactica* **16**: 327-329.

Buruchara, R.A. (1983) Determination of pathogenic variation in *Isariopsis griseola* sacc. and *Pseudomonas syringae* pv. *phaseolicola* (Burk., 1926) Young, Dye and Wilkie 1978. Ph.D. diss. University of Nairobi, Nairobi.

Cheng, G.Y., Legard, D.E., Hunter, J.E. & Burr, T.J. (1989) Modified bean pod assay to detect strains of *Pseudomonas syringae* pv. *syringae* that cause bacterial brown spot of snap bean. *Plant Disease* **73**: 419-423.

Edington, B.R. (1990) The identification of race 1 of *Pseudomonas syringae* pv. *phaseolicola* in South Africa. *Annual Report of the Bean Improvement Cooperative* **33**: 171.

Hale, C.N. & Taylor, J.D. (1973) Races of *Pseudomonas phaseolicola* causing halo blight of beans in New Zealand. *New Zealand Journal for Agricultural Research* **16**: 147-149.

Hildebrand, D.C., Schroth, M.C. & Sands, D.C. (1988) Pseudomonas. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed* (N.W. Schaad, ed) : 81-93. The American Phytopathological Society, St. Paul, MN, USA.

King, E.O., Ward, M.K. & Raney, D.E. (1954) Two simple media for demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**: 301-307.

Mabagala, R.B. & Saettler, A.W. (1992) Races and survival of *Pseudomonas syringae* pv. *phaseolicola* in Tanzania. *Plant Disease* **76**: 678-682.

Patel, P.N. & Walker, J.C. (1965) Resistance in *Phaseolus* to halo-blight. *Phytopathology* **55**: 889-894.

Saettler, A.W. (1991) Diseases caused by bacteria. In *Compendium of bean diseases* (R. Hall, ed) : 29-32. APS-Press, St. Paul, Minnesota.

Sleesman, J.P. & Leben, C. (1978) Preserving phytopathogenic bacteria at -70°C or with silica gel. *Plant Disease Reporter* **62**: 910-913.

Taylor, J.D. (1970) Bacteriophage and serological methods for the identification of *Pseudomonas phaseolicola* (Burkh.) Dowson. *Annals of Applied Biology* **66**: 387-395.

Taylor, J.D., Teverson D.M., Allen, M.A. & Pastor-Corrales, M.A. (1996) Identification

and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. *Plant Pathology* **45**: 469-478.

Teverson, D.M. (1991) Genetics of pathogenicity and resistance in the halo-blight disease of beans in Africa. Ph.D. diss. University of Birmingham, Birmingham.

Wharton, A.L. (1967) Detection of infection by *Pseudomonas phaseolicola* (Burkh.) Dowson in white-seeded dwarf bean seed stocks. *Annals of Applied Biology* **60**: 305-312.

Zaiter, H.Z.& Coyne, D.P. 1984. Testing inoculation methods and sources of resistance to the halo blight bacterium (*Pseudomonas syringae* pv. *phaseolicola*) in *Phaseolus vulgaris*. *Euphytica* **33**: 133-141.

Table 1. Origin of *Pseudomonas savastanoi* pv. *phaseolicola* isolates selected for race identification

Host	Locality		Number of isolates	Races detected
P. vulgaris	Gauteng:	Arnot	14	1,6,8
P. vulgaris		Bapsfontein	1	8
P. vulgaris, *P. coccineus*		Delmas	41	2,6,8
P. vulgaris, *P. coccineus*		Nigel	8	1,6,8
P. vulgaris, *P. coccineus*		Ogies	7	1,8
P. vulgaris		Pretoria	6	2,4,8
P. vulgaris	Free State:	Bethlehem	4	1
P. vulgaris		Bloemfontein	1	1
P. vulgaris		Bothaville	4	6
P. vulgaris		Bervie	1	1
P. vulgaris		Fouriesburg	1	8
P. vulgaris		Reitz	1	8
P. vulgaris		Warden	1	8
P. vulgaris		Kransfontein	4	1,6
P. vulgaris	Mpumalanga:	Burgershall	2	2,8
P. vulgaris, *P. coccineus*		Ermelo	39	1,2,6,8
P. vulgaris		Groblersdal	1	6
P. vulgaris, *P. coccineus*		Hendriena	5	1
P. vulgaris		Kendal	1	8
P. vulgaris, *P. coccineus*		Kriel	12	1,6,8,9
P. vulgaris, *P. coccineus*		Leslie	4	1,6
P. vulgaris, *P. coccineus*		Lothair	3	1
P. vulgaris		Lydenburg	1	6
P. vulgaris		Middelburg	5	1,2,8
P. vulgaris		Van Wyksdrif	1	8
P. vulgaris, *P. coccineus*		Vlakfontein	2	1,6
P. vulgaris		Komatipoort	9	1,6,8
P. vulgaris	Northwest:	Carletonville	5	1,2,8
P. vulgaris, *P. coccineus*, *P. lunatus*		Lichtenburg	7	6
P. vulgaris		Potchefstroom	10	1,8
P. vulgaris	KwaZulu\Natal:	Cedara	4	1,7
P. vulgaris		Greytown	12	6,7,8,9
P. vulgaris		Makatini	9	1
P. vulgaris		Normandien	2	6,8
P. vulgaris		Niekershoop	1	8
P. vulgaris	Cape Province:	Douglas	2	6
P. vulgaris		George	4	1,8
P. vulgaris		Klein Karoo	3	1
P. vulgaris		Kokstad	5	8
P. vulgaris		Kimberley	1	2

Table 2. Host range of *Pseudomonas savastanoi* pv. *phaseolicola* races in South Africa

Species	Cultivar	Races									
		1	2	3	4	5	6	7	8	9	Total
P. vulgaris:											
Large seeded:											
	Allubia Cerrillos								1		1
	And 888								1		1
	Atoki		1						1		2
	Bonus	23	1				5		7		36
	Breeding trials	1					9		16		26
	Broad acres						1				1
	Drakensberg						1	4			5
	Green beans	3	2				1		16		22
	Jenny						2				2
	Kid 27, 28, 35								3		3
	Kidney beans				1						1
	Leeukop								3		3
	Limpopo	1									1
	Montcalm								1		1
	Natal speckled sugar	2									2
	NCM 3031		1								1
	Pan 127								1		1
	Redlands Pioneer								1		1
	Sabie		1								1
	SSB 10, 30, 40	5	2				1		7		15
	Stormberg	4					1		3		8
	Stragonta						1				1
	SUG65,68,70,72								6		6
	Taylor								1		1
	Umlazi	2					2		1		5
	Umtata								1		1
	Wartburg	2	1				2		5	1	11
Small seeded:											
	Arctic		1				1		1		3
	Aurora						1		1		2
	Breeding trials		1						2		3
	CNC						1				1
	Coffee bean								1		1
	CSW 643								1		1
	Heuningberg		1								1
	Kamberg	1	1				3		3		8
	Kosi								2		2
	Mexico 235,309								2		2
	Mkuzi	1					1				2
	Nandi		1								1
	NEP 2								1		1
	Nuweveld								1		1
	Pan 122, 125								3		3
	Teebus						1		4		5
	Yellow haricot								1		1
P.coccineus:	Bomba						1				1
	Egyptian Great						1				1
	SSN1	20					4		5		29
P.lunatus			1								1
	CIAT Trials								4		4
	Mixed beans	1							3		4
	Unknown	3	1				3		8	3	18
TOTAL		69	16		1		43	4	118	4	255

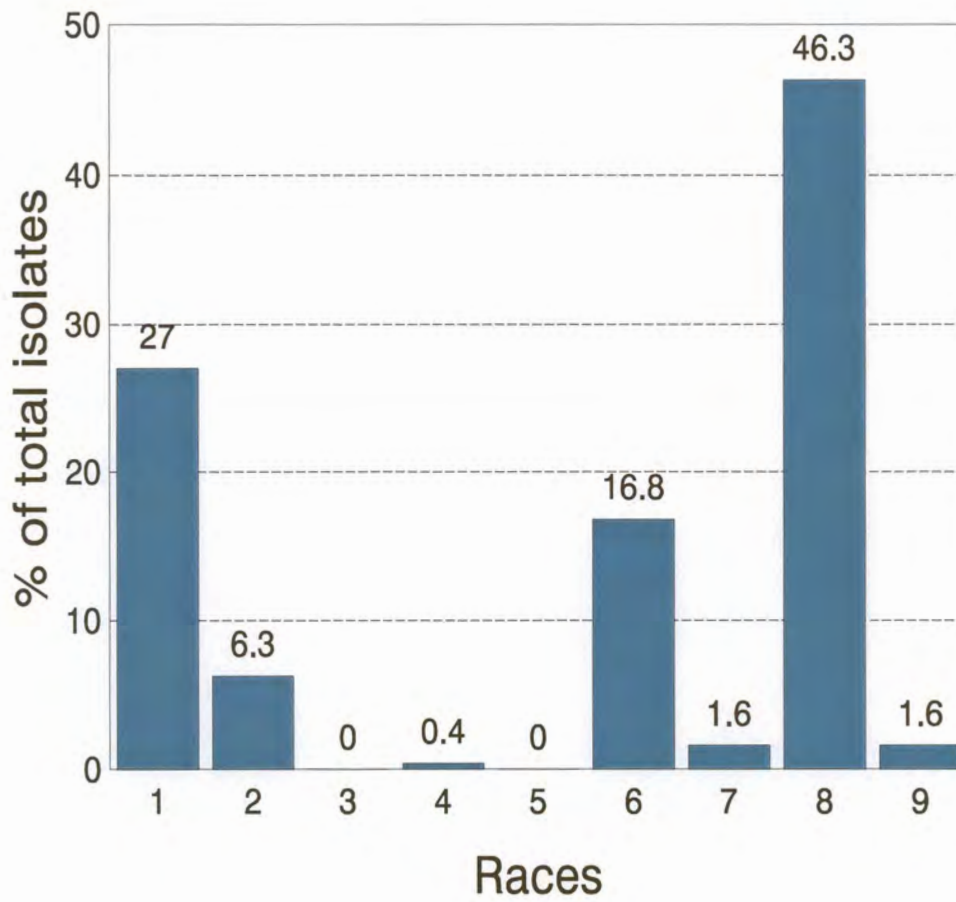

Figure 1. Races of *Pseudomonas savastanoi* pv. *phaseolicola* occurring on dry beans in South Africa

CHAPTER 4

PATHOGENIC AND GENETIC VARIATION IN *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* AND *X. AXONOPODIS* PV. *PHASEOLI* VAR. *FUSCANS* IN SOUTHERN AFRICA

ABSTRACT

One hundred and forty three common bacterial blight isolates from 44 localities in four countries, were inoculated onto eight *Phaseolus acutifolius* lines that differentiate between pathogenic races. This differential set was expanded to include resistant genotypes, XAN 159, GN #1 Nebr. sel 27, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 and cv. Teebus as susceptible check. Genetic variation within nine selected Xap and Xapf isolates and a non-pathogenic *Xanthomonas* isolate, was studied using RAPD and AFLP analysis. Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 were resistant to all isolates, while GN #1 Nebr. sel 27 and cv. Teebus were susceptible. Isolates varied in aggressiveness on cv. Teebus, however, pathogenic reaction on the set of differentials, indicated that all, but one isolate, grouped in what has been reported as race 2. Thus, results based on reaction of the majority isolates, suggest the absence of different races. However, the distinct differential reaction recorded for a single isolate, may prove to represent another, as yet unrecorded, race of this pathogen. Both RAPD and AFLP analyses revealed high frequency of DNA polymorphism among isolates and could distinguish between Xap, Xapf and a non-pathogenic isolate. Differences between Xap and Xapf isolates demonstrate that these are two distinct groups of bacteria. Information gained from

this study has enabled us to select the most appropriate isolates to use in a resistance breeding programme.

INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf), is a devastating seed-borne disease of dry beans (*Phaseolus vulgaris*) in many parts of the world (CIAT 1985). The disease is widespread throughout the South African production areas (Fourie 2002) and is favoured by high temperatures and high relative humidity (Sutton & Wallen 1970). In eastern and southern Africa common blight has been reported in 19 of the 20 bean producing countries (Allen 1995) and is considered one of five most important and widespread biotic constraints to dry bean production in sub-Saharan Africa (Gridley 1994). Genetic resistance is considered the most effective and economical strategy for the control of bean common blight (Rands & Brotherton 1925). However, deployment of resistance without knowledge of variation within a pathogen population could result in costly failure (Taylor *et al.* 1996).

Pathogenic variation in Xap and Xapf isolates has been demonstrated in several reports (Small & Worley 1956, Corey & Starr 1957, Schuster & Coyne 1971, Schuster *et al.* 1973, Yoshii *et al.* 1978, Schuster 1983, Jindal & Patel 1984). Ekpo & Saettler (1976) indicated that Xapf isolates were more pathogenic than Xap. These differences in pathogenicity have been confirmed by other investigators (Leakey 1973, Bozzano-Saguier & Rudolph 1994, Opio *et al.* 1996), but it has been suggested that the brown pigment is not associated with pathogenicity (Gilbertson *et*

al. 1991, Tarigan & Rudolph 1996) and should be considered of lesser pathological importance (Schuster & Coyne 1975).

Gilbertson *et al.* (1991) studied genetic diversity in isolates of Xap and Xapf, using DNA probes isolated from a single Xap isolate genome on isolates from different geographical locations. These studies indicated that there are two distinct groups of bacteria. However, similarities between isolates were revealed when probes were hybridised to DNA from other *X. campestris* pathovars, indicating sufficient similarity to consider Xapf a variety of Xap (Gilbertson *et al.* 1991).

Reports of physiological specialization in *P. vulgaris* have been contradictory. Zapata (1996) indicated *P. vulgaris* genotypes that are useful in differentiation of Xap. However, evidence exists suggesting quantitative interactions between Xap and *P. vulgaris* (Opio *et al.* 1996). Host specialization of Xap reactions on tepary *(P. acutifolius)* lines has been reported (Zapata & Vidaver 1987, Zaiter *et al.* 1989, Opio *et al.* 1996) with eight physiological races identified, suggesting a gene-for-gene relationship (Opio *et al.* 1996). Despite this gene-for-gene interaction, resistance to Xap and Xapf in *P. vulgaris*, derived from *P. acutifolius*, has remained non-specific and durable (Opio *et al.* 1996).

Tepary bean is an excellent source of resistance due to high resistance levels to Xap and Xapf. Variation that may exist in the local pathogen population is important when selecting parents with resistance originating from tepary cultivars. The aim of the study was to determine pathogenic and genetic variation in Xap and Xapf isolates in southern Africa ensuring that appropriate resistance sources are deployed when developing CBB resistant cultivars.

MATERIAL AND METHODS

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Isolation and identification of isolates

Diseased plant material was collected from major bean production areas in South Africa and Malawi, Lesotho and Zimbabwe. Leaves were rinsed under running tap water for 10 min, surface-disinfested for 3 min in 3.5% sodium hypochlorite and then rinsed twice in sterile water for 1 min each. Leaf material was macerated in a droplet of sterile water and streaked onto yeast-extract-dextrose-calcium-carbonate (YDC) agar (Schaad & Stall 1988). Plates were incubated at 25°C. Following 72 hr incubation, yellow-pigmented colonies typical of *Xanthomonas* spp. were purified on YDC agar by a series of single colony transfers. Production of brown diffusible pigment on YDC differentiated Xapf from Xap isolates (Basu & Wallen 1967). Agglutination of antiserum specific to Xap and Xapf, obtained from Adgen Agrifood Diagnostics, Auchincruive, Scotland, was used to identify isolates. Pathogenicity tests on susceptible cultivar Teebus were done to confirm identity of isolates.

Pathogenicity tests

Seed from eight tepary lines previously reported to differentiate between Xap and Xapf races (Table 1) (Opio *et al.*, 1996), were obtained from Dr. DP Coyne, University of Nebraska, Lincoln, USA and multiplied from a single seed in a greenhouse to ensure genetically uniform material. The tepary differential set was expanded to include resistant genotypes, XAN 159, GN #1 Nebr. sel 27, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6. Resistance in these lines are all tepary derived. Cultivar Teebus was included as susceptible check. Five seeds of each genotype were planted in 15-cm-diameter plastic pots in sterile soil and maintained in a greenhouse

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at 18°C night/28°C day. Seedlings were thinned to four plants per pot after emergence. One pot per differential was used per isolate, each plant representing a replicate. Pots were randomised prior to inoculation. Experiments were repeated to confirm reactions of isolates.

One hundred and forty three isolates from 44 localities in four countries of southern Africa were selected for the study (Table 2). Four isolates received from the International Centre for Agriculture in the Tropics (CIAT) were included as reference cultures. Isolates used for each experiment were regenerated from storage at -72 °C, because loss of pathogenicity was encountered by sub-culturing. Inoculum was prepared by suspending 48- to 72-h-old cultures in sterile distilled water and adjusting it turbidimetrically to contain approximately 10^8 CFU/ml. Fourteen to 20-day-old plants with fully expanded first trifoliate leaves were used for inoculation. Plants were inoculated using the multiple-needle inoculation method (Andrus 1948). Control plants were inoculated with sterile distilled water. Inoculated plants were kept in a greenhouse at 18°C night/28°C day. Plants were rated for infection 14 days after inoculation on a 1 to 9 scale (Aggour *et al.* 1989). Plants, rated 1 to 3, were classified as resistant (incompatible) and ratings of 4 to 9 considered susceptible (compatible).

Isolation of bacterial DNA

Eight isolates (two Xap and six Xapf) from southern Africa, one Xapf isolate from CIAT and a non-pathogenic *Xanthomonas* isolate (Table 3) were used in genetic studies. These isolates were selected based on their geographic origin. Isolates were cultured in 50 ml nutrient broth for 24-48 hr at 25°C prior to DNA isolation.

Bacterial cells were collected by centrifugation at 5 000 rpm for 10 min. Cells were washed three times by resuspending in 5 ml 1 M NaCl and centrifugation at 5 000 rpm for 10 min, followed by two wash steps in 5 ml sterile distilled water. Washed cells were resuspended in 10 ml warm (55°C) extraction buffer containing 0.2 M Tris.HCl (tris (hydroxymethyl) aminomethane), pH 8.0; 10 mM EDTA (ethylenediaminetetraacetate), pH 8.0; 0.5 M NaCl; 1% (w/v) SDS (sodiumdodecylsulfate) and 10 $\mu g.ml^{-1}$ Proteinase K. Resuspended cells were incubated in a water bath at 55°C for one hr and half a volume 7.5 M ammonium acetate was added. The suspension was mixed by gentle inversion and incubated at room temperature for 10 min. Phase separation was enhanced by adding 100 μl TE buffer (10 mM Tris.HCl, pH 8.0; 1 mM EDTA, pH 8.0). Phases were separated by centrifugation at 14 000 rpm for 15 min. The upper aqueous layer was transferred to a fresh tube containing an equal volume of isopropanol, mixed by gentle inversion and incubated at room temperature for a minimum of 2 hr to overnight. DNA was collected by centrifugation at 14 000 rpm for 15 min. The precipitated DNA was washed twice in 1 ml ice-cold 70% (v/v) ethanol, the pellet air-dried at room temperature, and resuspended in 10 μl TE buffer. The DNA was treated with RNase for two hours at 37°C and concentration and purity estimated by measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ng/μl.

RAPD analysis

Arbitrary 10 bp oligonucleotide primers (Operon Technologies, Table 4) were used for the polymerase chain reaction (PCR) based on the protocol of Williams *et al.*

(1990), with minor modifications. Amplification reactions were performed in a 25 μl reaction volume containing Promega (Promega Corporation, Madison, Wisconsin) reaction buffer (500 mM KCl; 100 mM Tris.HCl, pH 9.0 at 25°C; 1% (v/v) Triton X-100), 2 mM $MgCl_2$, 100 μM of each dNTP (dATP, dCTP, dGTP and dTTP), 5 pmol primer, 0.5 units *Taq* DNA polymerase (Promega) and 25 ng template DNA. Reactions were performed using a Hybaid Thermal Cycler (Hybaid Limited, UK) programmed for 5 min at 95°C, 55 cycles of 1 min at 95°C, 1.5 min at 35°C, and 2.5 min at 72°C, followed by one cycle of 5 min at 72.5°C and 5 min at 28°C. The amplification products were analysed by electrophoresis on 1.5% (w/v) agarose gels (Seakem LE) at 80V for 2 hr using UNTAN buffer (0.4 M Trisbase; 0.02 M EDTA, pH 7.4) and detected by staining with 1 $\mu g.ml^{-1}$ ethidium bromide. Gels were photographed under UV light with polaroid 667 film. All reactions were repeated and only reproducible bands were considered in this study.

AFLP analysis

AFLP adapters and primers were designed based on the method of Vos *et al.* (1995). Adapter and primer sequences are given in Table 5. Primers were synthesised by GibcoBRL (Life Technologies, Glasgow, United Kingdom) and oligonucleotides used for the adapters were PAGE (polyacrylamide gel electrophoresis) purified. Adapters were prepared by adding equimolar amounts of both strands, heating for 10 min to 65°C in a water bath and leaving it to cool down to room temperature.

AFLP analysis was performed following the protocol described by Vos *et al.* (1995) and the product manual supplied by Life Technologies Inc. (Glasgow, UK), with minor modifications. Restriction enzymes *Eco*RI and *Mse*I were used to digest

500 ng of isolate genomic DNA for 4 hr and the reaction mixture, without inactivation of the restriction endonucleases, was subjected to the overnight ligation of adapters at 37°C, followed by pre-amplification. The ligation mixture was not diluted prior to pre-amplification and the pre-amplification DNA was diluted only 1:5 prior to selective amplification. The selective amplification was conducted using two primers, and the *Mse*I primers always had three selective nucleotides while the *Eco*RI primers had two, three or four selective nucleotides (Table 5).

Gel electrophoresis

Gel electrophoresis for AFLP analysis was performed using the protocol of Vos *et al.* (1995) but employing a 5% (w/v) denaturing polyacrylamide gel (19:1 acrylamide: bis-acrylamide; 7 M urea; 1x TBE buffer (89 mM Tris-borate; 2.5 mM EDTA)). Electrophoresis was performed at constant power, 80 W for approximately 2 hr.

Silver staining for DNA visualisation

Polyacrylamide gels were silver-stained following the protocol described by the Silver Sequence™ DNA Sequencing System manual supplied by Promega (Madison, WI, USA). The gels were left upright overnight to air dry and photographed by exposing photographic paper (Kodak Polymax II RC) directly under the gel to about 20 sec of dim light. This produced a negative image, exactly the same size of the gel.

Statistical analyses

Data obtained from RAPD and AFLP analysis on ten isolates were used for statistical analysis. DNA bands obtained for each isolate were scored based on their presence (1) or absence (0). Only reliable and repeatable bands were considered. Pair wise genetic distances were calculated between isolates Nei and Li (1979). Cluster analysis was done by the unweighed paired group method using arithmetic averages (UPGMA). All calculations were done with the aid of the programme NTSYSpc version 2.02i.

RESULTS

Identification of isolates

All isolates collected (except Z93) were identified as Xap and Xapf on the basis of their agglutination of specific antiserum and pathogenicity on cv. Teebus (Table 2). Isolate, Z93 did not induce any disease on cv. Teebus and exhibited a weak reaction when tested with the antiserum. The majority of isolates (72%) produced a brown diffusible pigment on YDC agar and were classified as Xapf. Differences in aggressiveness between isolates on the cv. Teebus were detected with mean ratings ranging from moderately to highly susceptible (5-9). The most aggressive isolates included both Xap and Xapf.

Pathogenicity tests

All isolates inoculated onto the tepary differential set induced reaction on genotype Nebr. #21. The majority of isolates (99,3%) exhibited an incompatible reaction

(rating 1-3) on the remaining genotypes, resembling the infection pattern of race 2 (Opio *et al.* 1996) (Table 1). One isolate (X539) induced disease (mean ratings 4-9) on all tepary genotypes and did not resemble any infection pattern previously reported (Table 1). A small percentage of isolates induced a slight reaction on genotypes Nebr. #1 (6.3%; rating=1-2.25), Nebr. #5 (1.4%; rating=1-2.3), Nebr. #8b (9.1%; rating=1-2.0), Nebr. #19 (1.4%; rating=1-1.5), PI 321638 (23.1%; rating=1-2.8) and L242-45 (4.2%; rating=1-1.5). These reactions were not repeatable in further experiments and reactions were, therefore, considered incompatible with mean ratings not exceeding 3. No symptoms developed on Nebr. #22 except when inoculated with isolate X539. Teebus was susceptible to all the isolates tested except for one non-pathogenic isolate (Z93) that did not induce disease on any of the inoculated lines.

Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 were generally resistant to all isolates (mean rating=1-3). Six isolates (X563, X573, X121, X295, X561 and X594) induced disease on XAN 159 with a mean rating of 4. GN #1 Nebr. sel 27 were susceptible to all isolates (mean rating=7).

RAPD analysis

RAPD analysis produced between two and ten fragments (Fig. 1), but results were not repeatable. Best results were obtained with primer OPA-02. RAPD analysis revealed a high frequency of DNA polymorphism among isolates and were able to distinguish between Xap, Xapf and the non-pathogenic isolate.

AFLP analysis

The AFLP fingerprinting techniques revealed complex banding patterns that were difficult to interpret (Fig. 2). DNA fingerprinting techniques revealed a high frequency of DNA polymorphism among isolates with a low presence of shared fragments between isolates (Fig. 2). A total of 756 fragments were amplified using 16 primer pair combinations. Only 2.64% of these fragments were shared between all ten isolates. Primer combinations varied in their ability to detect polymorphisms, ranging from 16 to 86 polymorphisms per primer pair, with an average of 47.3 fragments per primer combination. Fragment sizes varied between 100 and 900 base pairs. Selectivity of AFLP analysis, using two restriction enzymes, was enhanced, by using primers containing two, three or four selective nucleotides. This enhancement of primer selectivity did not reduce the complexity of resulting AFLP banding patterns. Best results were obtained when primers containing three selective nucleotides were used in the AFLP analysis.

As with RAPD analysis, the AFLP technique also separated Xap, Xapf and the non-pathogenic isolate into different groups. Fingerprinting techniques, thus, clearly differentiated amongst Xap as well as Xapf isolates. Combined data produced by RAPD and AFLP techniques are shown in Fig. 3. The phenogram drawn using pooled data from the RAPD and AFLP analysis (Fig. 3), showed a maximum similarity between any two isolates of 81% (Xapf isolates Les19 and Xapf180). The minimum similarity between any two isolates was 67.5% (Xap isolates X448 and X590). The Xapf cluster of isolates was linked to the Xap cluster of isolates at a similarity of 45.6% and the non-pathogenic isolate Z93 was linked to the Xapf/Xap cluster with a similarity of 30.6%. Isolates within the Xapf cluster exhibited a similarity of 71%. The obtained cophenetic correlation value of r=0.994 indicated that the

UPGMA cluster analysis was statistically significant.

DISCUSSION

Results of this study, based on pathogenicity and molecular characterizations, showed that diversity exists within Xap(f) populations, in southern Africa. Isolates differed in production of brown pigment as well as aggressiveness on the cv. Teebus. Although it has previously been reported that pigment producing Xapf isolates are more aggressive (Leakey 1973, Ekpo & Saettler 1976, Bozzano-Saguier & Rudolph 1994, Opio *et al.* 1996), the most aggressive isolates in this study included both Xap and Xapf. Isolates with lower levels of aggressiveness, however, belonged to Xap (rating on cv Teebus=5). Gilbertson *et al.* (1991) and Tarigan & Rudolph (1996) reported that pigment is not associated with pathogenicity and should be considered of little pathological importance (Schuster & Coyne 1975). Although no differences in disease reaction were observed, RAPD and AFLP analyses demonstrated that Xap and Xapf represent two distinct groups of bacteria. Although widely distributed in Africa (Opio et al. 1996, Fourie 2002), Xapf isolates do not occur in Costa Rica, Caribbean countries and Spain (CIAT 1992, C. Assensio, MBG-CSIC; personal communication).

All isolates (except X539 and Z93) inoculated on the tepary differential set had an identical infection pattern, similar to race 2 following the classification of Opio *et al.* (1996). Although a number of isolates induced only a mild reaction on some of the tepary lines, these reactions were not always repeatable, which is similar to results obtained by Zaiter *et al.* (1989). The non-pathogenic isolate (Z93) did not induce disease on any of the lines tested.

Except for isolate X539, which exhibited a significantly different infection pattern, no races other than race 2, previously described by Opio *et al.* (1996), could be distinguished. The distinct pattern of differential reaction recorded for this isolate, may represent another, as yet unrecorded, race of Xap. The possibility exists that isolates identical to X539 exist, but may not have been sampled in this study. Continuous monitoring of CBB isolates in future is necessary in order to detect presence of isolates exhibiting differential reactions. Although isolate X539 was pathogenic on the eight tepary lines tested, no disease developed on resistant genotypes used to supplement the differential set, except for GN #1 Nebr. sel. 27. Using these resistant genotypes in a resistance breeding programme would, therefore, not be influenced by the occurrence of this isolate.

Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 that were used to supplement the tepary differential set, were generally resistant to all isolates tested. Resistance in all these lines is tepary-derived. XAN 159 was slightly susceptible to a small number of isolates. Resistance instabilities such as these have been reported previously in XAN 159 and its progeny (Beebe & Pastor-Corrales 1991), however, it is still widely used in resistance breeding programmes (Beebe & Pastor-Corrales 1991, Fourie & Herselman 2002, Park *et al.* 1998, Mutlu *et al.* 1999, Singh & Muñoz 1999).

The reportedly resistant line GN #1 Nebr. sel 27 was susceptible to all the isolates used in this study. This line was originally derived from inter-specific crosses between *P. vulgaris* and *P. acutifolius* and has been used in many breeding programmes as a source of resistance (Coyne & Schuster 1974, Mohan & Mohan 1983). Recent molecular studies have, however, indicated that resistance in GN #1 Nebr. sel 27 is derived from *P. vulgaris* and not *P. acutifolius*, as previously described

(Miklas *et al.* 2002). Although susceptible in South Africa, GN #1 Nebr. sel 27 and lines derived from it, have tested resistant in some parts of the USA (Coyne & Schuster 1974) and Spain (C. Assensio, MBG-CSIC: personal communication). Inconsistency in these results could have resulted from the limited distribution of Xapf in some areas of the USA and Spain (R. Gilbertson, University of California-Davis: personal communication).

Results of DNA fingerprinting techniques indicated that genetic diversity exists among isolates of the common blight pathogen. Differences between Xap and Xapf isolates show that these represent two distinct groups of bacteria. Similar distinction between these two groups was also reported by Gilbertson *et al.* (1991), using RFLP's. Non-pathogenic *Xanthomonas* commonly associated with beans could be distinguished from Xap and Xapf using both RAPD and AFLP techniques. These results are similar to those of Gilbertson *et al.* (1990) who distinguished between non-pathogenic and pathogenic isolates using RFLP's.

Although isolate X539 gave a significantly different infection pattern when inoculated onto the tepary lines, no significant difference between this isolate and the others Xapf isolates could be detected using different molecular techniques. It has been reported that strains of Xap and Xapf from similar geographic locations had similar, but not identical RFLP patterns (Gilbertson *et al.* 1991, CIAT 1992). This could not be confirmed in the present, study and is possibly due to the small number of isolates tested.

Results obtained in this study indicate that both pathogenic and genetic variation exist in the CBB pathogen population in southern Africa. However, identical reactions with the majority of isolates on the tepary lines, showed that different CBB races do not occur. Information gained from this study made it possible to select the

most appropriate isolates to use in a resistance breeding programme.

REFERENCES

Aggour, A.R., Coyne, D.P. & Vidaver, A.K. (1989) Comparison of leaf and pod disease reactions of beans (*Phaseolus vulgaris* L.) inoculated by different methods with strains of *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. *Euphytica* **43**: 143-152.

Allen, D.J. (1995) An annotated list of diseases, pathogens and associated fungi of the common bean (*Phaseolus vulgaris*) in Eastern and Southern Africa. *Phytopathological Papers* **34**. CAB International/Centro Internacional de Agricultura Tropical, Cali, Colombia.

Andrus, C.F. (1948) A method of testing beans for resistance to bacterial blights. *Phytopathology* **38**: 757-759.

Basu, P.K. & Wallen, V.R. (1967) Factors affecting virulence and pigment production of *Xanthomonas phaseoli* var. *fuscans*. *Canadian Journal of Botany* **45**: 2367-2374.

Beebe, S.E. & Pastor-Corrales, M.A. (1991) Breeding for disease resistance. In *Common Beans, Research for Crop Improvement* (A. Van Schoonhoven & O. Voysest, eds) : 561-610. CAB International, Wallingford.

Bozzano-Saguier, G. & Rudolph, K. (1994) Differential reactions of bush bean cultivars towards common and fuscous blight (*Xanthomonas campestris* pv. *phaseoli* and *X. c. phaseoli* var. *fuscans*). *Annual Report of the Bean Improvement Cooperative* **37**: 227-228.

CIAT. (1985) Bean Programme Annual Report for 1985. Centro Internacional de

Agricultura Tropical, Cali, Colombia.

CIAT. (1992) Bean Programme Annual Report for 1992. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Corey, R.R. & Starr, M.P. (1957) Colony types of *Xanthomonas phaseoli*. *Journal of Bacteriology* **74**: 137-140.

Coyne, D.P. & Schuster, M.L. (1974) Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. *Euphytica* **23**: 651-656.

Ekpo, E.J.A. & Saettler, A.W. (1976) Pathogenic variation in *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans*. *Plant Disease Reporter* **60**: 80-83.

Fourie, D. (2002) Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology* **150**: 220-226.

Fourie, D. & Herselman, L. (2002) Breeding for common blight resistance in dry beans in South Africa. *Annual Report of the Bean Improvement Cooperative* **45**: 50-51.

Gilbertson, R.L., Rand, R.E. & Hagedorn, D.J. (1990) Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. *Plant Disease* **74**: 322-327.

Gilbertson, R.L., Otoya, M.M., Pastor-Corrales, M.A. & Maxwell, D.P. (1991) Genetic diversity in common blight bacteria is revealed by cloned repetitive DNA sequences. *Annual Report of the Bean Improvement Cooperative* **34**: 37-38.

Gridley, H.E. (1994) Bean production constraints in Africa with special reference to breeding for resistance to bean common mosaic virus in Uganda. In *Proceedings of a Pan-African working group meeting on bacterial and virus*

diseases of common bean. CIAT African Workshop Series (D.J. Allen & R.A. Buruchara, eds) **34**: 33-39. Kampala, Uganda.

Jindal, J.K. & Patel, P.N. (1984) Variability in Xanthomonads in grain legumes. IV. Variations in bacteriological properties of 83 isolates and pathogenic behaviour of cultural variants. *Phytopathology* **110**: 63-68.

Leakey, C.L.A. (1973) A note on *Xanthomonas* blight of beans (*Phaseolus vulgaris* (L.) savi) and prospects for its control by breeding for tolerance. *Euphytica* **22**: 132-140.

Miklas, P.N., Coyne, D.P., Grafton, K.F., Mutlu, N., Reiser, J. & Singh, S.P. (2002) A major QTL for common bacterial blight resistance in GN No.1 Sel.27 derives from the great northen landrace Montana No. 5 and not tepary bean. *Annual Report of the Bean Improvement Cooperative* **45**: (in press).

Mohan, S.T. & Mohan, S.K. (1983) Breeding for common bacterial blight resistance in beans. *Annual Report of the Bean Improvement Cooperative* **26**: 14-15.

Mutlu, N., Coyne, D.P., Park, S.O., Steadman, J.R., Reiser, J. & Jung, G. (1999) Backcross breeding with RAPD molecular markers to enhance resistance to common bacterial blight in pinto beans. *Annual Report of the Bean Improvement Cooperative* **42**: 7-8.

Nei, M. & Li. W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science, USA* **76**: 5269-5273.

Opio, A.F., Allen, D.J. & Teri, J.M. (1996) Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathology* **45**: 1126-1133.

Park, S.J., Michaels, T.E. & Dhanvantari, B.N. (1998) Breeding for resistance to

common bacterial blight and its effect on agronomic performance and processing quality in dry bean. *Annual Report of the Bean Improvement Cooperative* **41**: 25-26.

Rands, R.D. & Brotherton, W. (1925) Bean varietal tests for disease resistance. *Journal of Agricultural Research* **31**: 110-154.

Schaad, N.W. & Stall R.E. (1988) Xanthomonas. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria 2nd ed* (N.W. Schaad, ed) : 81-93. The American Phytopathological Society, St. Paul, MN, USA.

Schuster, M.L. & Coyne, D.P. (1971) New virulent strains of *Xanthomonas phaseoli*. *Plant Disease Reporter* **55**: 505-506.

Schuster, M.L., Coyne, D.P. & Hoff, B. (1973) Comparative virulence of *Xanthomonas phaseoli* strains from Uganda, Colombia and Nebraska. *Plant Disease Reporter* **57**: 74-75.

Schuster, M.L. & Coyne, D.P. (1975) Genetic variation in bean bacterial pathogens. *Euphytica* **24**: 143-147.

Schuster, M.L. (1983) Variability in virulence of Dominican Republic *Xanthomonas phaseoli* in CIAT *Phaseolus vulgaris* cultivars. *Fitopatologia Brasileira* **8**: 339-345.

Singh, S.P. & Muñoz, C.G. (1999) Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Science* **39**: 80-89.

Small, B.C. & Worley, J.F. (1956) Evaluation of 2,3,5-triphenyl tetrazolium chloride for obtaining pathogenic types from stock cultures of halo blight and common blight organisms. *Plant Disease Reporter* **40**: 628.

Sutton, M.D. & Wallen, V.R. (1970) Epidemiological and ecological relations of *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* on beans in southwestern

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YUNIBESITHI YA PRETORIA

Ontario, 1961-1968. *Canadian Journal of Botany* **48**: 1329-1334.

Tarigan, J.R. & Rudolph, K. (1996) Investigations on the resistance of bean genotypes to *Xanthomonas campestris* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* and on the differentiation of bacterial strains. *Annual Report of the Bean Improvement Cooperative* **39**: 284-285.

Taylor, J.D., Teverson, D.M., Allen, M.A. & Pastor-Corrales, M.A, (1996) Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. *Plant Pathology* **45**: 469-478.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van Der Lee, T., Hornes, M., Freijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995) AFLP: a new concept for DNA fingerprinting. *Nucleic Acids Research* **21**: 4407-4414.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Yoshii, K., Gálvez-E, G.E. & Alvarez-A, G. (1978) Screening bean germplasm for tolerance to common blight caused by *Xanthomonas phaseoli* and the importance of pathogenic variation to varietal improvement. *Plant Disease Reporter* **62**: 343-347.

Zaiter, H.Z., Coyne, D.P., Vidaver, A.K. & Steadman, J.R. (1989) Differential reaction of tepary bean lines to *Xanthomonas campestris* pv. *phaseoli*. *Horticultural Science* **24**: 134-137.

Zapata, H.Z. & Vidaver, A.K. (1987) Differentiation of *Xanthomonas campestris* pv. *phaseoli* into pathogenic races based on the tepary bean reactions. *Phytopathology* **77**: 1709 (Abstract).

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Zapata, M. (1996) Pathogenic variability of *Xanthomonas campestris* pv. *phaseoli*. *Annual Report of the Bean Improvement Cooperative* **39**: 136-137.

Table 1. Interaction of *Xanthomonas axonopodis* pv. *phaseoli* and *P. acutifolius* (Opio *et al.* 1996)

Race	Nebr.#1	Nebr.#5	Nebr.#8b	Nebr.#19	Nebr.#21	Nebr.#22	PI321638	L242-45
1	-	-	-	-	-	-	+	-
2	-	-	-	-	+	-	-	-
3	+	-	-	-	-	-	-	-
4	-	+	-	+	+	-	+	-
5	-	-	-	-	+	-	+	-
6	-	+	-	-	+	+	+	-
7	-	-	-	+	+	-	-	-
8	-	-	-	-	-	-	-	-
X539	+	+	+	+	+	+	+	+

-, incompatible reaction (resistant); +, compatible reaction (susceptible)

Table 2. Origin and host range of *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* isolates used for pathogenicity tests on the dry bean cv. Teebus

Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X6	Cedara	Unknown	+	Xap	9
X78	Kriel	Unknown	+	Xapf	9
X101	M.Hill	Unknown	+	Xapf	9
X102	M.Hill	Unknown	+	Xapf	7
X105	M.Hill	Unknown	+	Xapf	9
X110	M.Hill	Unknown	+	Xapf	9
X111	Unknown	Mixture	+	Xap	9
X117	Unknown	Mixture	+	Xap	9
X119	Unknown	Mixture	+	Xapf	7
X120	Unknown	Mixture	+	Xapf	9
X121	Unknown	Mixture	+	Xap	8
X122	Unknown	Mixture	+	Xap	9
X125	Unknown	Mixture	+	Xapf	9
X130	Ermelo	Kamberg	+	Xapf	8
X138	Kokstad	Helderberg	+	Xapf	9
X147	Carletonville	Redlands Pioneer	+	Xapf	7
X172	Potchefstroom	SSB 30	+	Xapf	9
X176	Potchefstroom	MCM 3031	+	Xap	7
X180	Carletonville	Nep 2	+	Xapf	9
X185	Carletonville	Nep 2	+	Xapf	9
X186	Carletonville	S 1051	+	Xapf	9
X188	Carletonville	S 1051	+	Xapf	9
X189	Carletonville	CNC	+	Xapf	9
X193	Delmas	Breeding material	+	Xap	9
X195	Cedara	Breeding material	+	Xapf	9
X200	Cedara	Breeding material	+	Xapf	9
X206	Delmas	Kamberg	+	Xapf	9
X208	Ogies	Wartburg	+	Xap	9
X214	Ermelo	Teebus	+	Xapf	9
X216	Ermelo	Teebus	+	Xapf	8
X229	Cedara	Breeding material	+	Xapf	9
X231	Bergville	Broad Acres	+	Xap	9
X253	Greytown	Drakensberg	+	Xapf	8
X261	Dundee	Jenny	+	Xapf	9
X269	Lichtenburg	Jenny	+	Xapf	9
X275	Greytown	Drakensberg	+	Xap	7
X277	Delmas	Jenny	+	Xapf	9
X279	Ukulinga	Drakensberg	+	Xapf	6
X280	Ukulinga	Drakensberg	+	Xapf	8
X285	Delmas	Helderberg	+	Xapf	7
X288	Kransfontein	Bonus	+	Xapf	9
X289	Kransfontein	Bonus	+	Xapf	9
X290	Kransfontein	Bonus	+	Xapf	9
X291	Kransfontein	Broad Acres	+	Xapf	9
X292	Kransfontein	Broad Acres	+	Xapf	9
X293	Kransfontein	Broad Acres	+	Xapf	9
X294	Kransfontein	Bonus	+	Xapf	8
X295	Bethlehem	Mixture	+	Xap	5

Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X318	Kransfontein	Bonus	+	Xapf	8
X322	Douglas	Kamberg	+	Xapf	9
X323	Douglas	Kamberg	+	Xapf	9
X324	Douglas	Kamberg	+	Xapf	8
X335	Derby	PAN 143	+	Xapf	8
X337	Dundee	Sabie	+	Xapf	8
X338	Carletonville	Drakensberg	+	Xap	9
X339	Carletonville	Drakensberg	+	Xap	9
X341	Carletonville	Drakensberg	+	Xap	9
X346	Rietgat	SSN 1	+	Xap	9
X350	Kroonstad	Bonus	+	Xapf	9
X359	Reitz	Limpopo	+	Xap	9
X409	Chrissiesmeer	Helderberg	+	Xapf	9
X410	Chrissiesmeer	Helderberg	+	Xapf	8
X414	Chrissiesmeer	Breeding material	+	Xapf	8
X421	Winterton	Kranskop	+	Xapf	8
X423	Winterton	Kranskop	+	Xapf	7
X424	Ermelo	Kranskop	+	Xapf	9
X426	Middelrus	Kranskop	+	Xapf	7
X428	Cyferbult	Kranskop	+	Xapf	8
X443	Carletonville	Unknown	+	Xap	8
X445	Carletonville	Breeding material	+	Xapf	8
X446	Carletonville	Breeding material	+	Xap	9
X447	Amersfoort	Kamberg	+	Xapf	9
X448	Wildebeestfontein	Helderberg	+	Xap	9
X451	Cyferbult	Helderberg	+	Xap	9
X457	Cedara	Breeding material	+	Xapf	8
X458	Cedara	Breeding material	+	Xapf	8
X459	Cedara	Breeding material	+	Xapf	9
X460	Cedara	Breeding material	+	Xapf	9
X462	Vivo	Kranskop	+	Xapf	9
X464	Vivo	Kranskop	+	Xapf	8
X470	Vivo	Kranskop	+	Xapf	8
X471	Vivo	Kranskop	+	Xapf	8
X472	Tom Burke	Kranskop	+	Xapf	8
X473	Pietersburg	Kranskop	+	Xapf	8
X474	Cedara	Breeding material	+	Xapf	7
X476	Lichtenburg	Kranskop	+	Xapf	8
X487	Vivo	Kranskop	+	Xapf	9
X492	Tom Burke	Kranskop	+	Xapf	9
X496	Pietersburg	Kranskop	+	Xap	7
X498	Vivo	Kranskop	+	Xapf	8
X505	Ellisras	Unknown	+	Xap	9
X510	Dendron	Teebus	+	Xapf	8
X513	Dendron	Kranskop	+	Xapf	9
X520	Grootpan	Unknown	+	Xap	5
X521	Koster	Unknown	+	Xapf	9
X522	Greytown	PAN 146	+	Xapf	9
X523	Cedara	Breeding material	+	Xapf	7
X524	Clarens	Unknown	+	Xapf	7
X526	Bethlehem	Leeukop	+	Xapf	8

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Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X527	Bethlehem	Bonus	+	Xap	9
X528	Clarens	Unknown	+	Xapf	9
X530	Bethlehem	Bonus	+	Xapf	8
X532	Delmas	Teebus	+	Xap	9
X534	Koster	Unknown	+	Xapf	8
X539	Ermelo	Unknown	+	Xapf	9
X551	Delmas	Kranskop	+	Xapf	9
X552	Delmas	Kranskop	+	Xapf	8
X553	Delmas	Kranskop	+	Xapf	8
X555	Reitz	Kranskop	+	Xapf	8
X559	Bergville	Volunteer beans	+	Xapf	8
X561	Clocolan	PAN 148	+	Xap	7
X562	Clocolan	PAN 148	+	Xap	7
X563	Clocolan	Kranskop	+	Xap	7
X565	Clocolan	Sabie	+	Xapf	9
X569	Greytown	Mkuzi	+	Xapf	8
X573	Delmas	Kranskop	+	Xap	5
X576	Newcastle	Sabie	+	Xapf	8
X578	Clocolan	Sabie	+	Xapf	6
X579	Clocolan	Sabie	+	Xapf	9
X586	Fouriesburg	PAN 148	+	Xap	9
X594	Fouriesburg	Kranskop	+	Xap	7
X598	Fouriesburg	Stormberg	+	Xap	9
X602	Keiskammahoek	Kranskop	+	Xap	9
X604	Keiskammahoek	Kranskop	+	Xap	9
X610	Dohne	Helderberg	+	Xapf	9
X618	Potchefstroom	Unknown	+	Xapf	8
XCP123	CIAT	Unknown	+	Xap	9
XCPF174	CIAT	Unknown	+	Xapf	9
XCPF180	CIAT	Unknown	+	Xapf	9
XCP183	CIAT	Unknown	+	Xap	9
Z93	Zimbabwe	Unknown	(+)	Xanthomonas	1
Z328	Zimbabwe	Unknown	+	Xap	7
Z332	Zimbabwe	Unknown	+	Xap	8
LES2	Lesotho	Unknown	+	Xapf	9
LES6	Lesotho	Unknown	+	Xapf	8
LES11/00	Lesotho	Unknown	+	Xapf	8
LES13	Lesotho	Unknown	+	Xapf	7
LES16/00	Lesotho	Unknown	+	Xapf	7
LES19/00	Lesotho	Unknown	+	Xapf	9
LES54/00	Lesotho	Unknown	+	Xapf	8
MAL13	Malawi	Unknown	+	Xap	8
MAL15	Malawi	Unknown	+	Xapf	7
MAL38	Malawi	Unknown	+	Xap	7
MAL61	Malawi	Unknown	+	Xapf	8

Table 3. Bacterial isolates used for RAPD and AFLP to study genetic variation

Isolate no	Xap/Xapf	Locality
X448	Xap	Wildebeestfontein, SA
X590	Xap	Fouriesburg, SA
Z93	Xanthomonas	Zimbabwe
X279	Xapf	Ukulinga, SA
X462	Xapf	Vivo, SA
X521	Xapf	Koster, SA
X539	Xapf	Ermelo, SA
Les19	Xapf	Lesotho
Mal61	Xapf	Malawi
Xapf180	Xapf	CIAT, Colombia

Table 4. Primer sequences used for RAPD analysis in genetic variation studies of Xap and Xapf

Name	Sequence (5'-3')
OPA-02	TGCCGAGCTG
OPA-07	GAAACGGGTG
OPA-09	GGGTAACGCC
OPA-18	AGGTGACCGT
OPD-01	ACCGCGAAGG
OPD-02	GGACCCAACC
OPD-03	GTCGCCGTCA
OPD-04	TCTGGTGAGG
OPG-08	TCACGTCCAC
OPG-10	AGGGCCGTCT
OPS-01	CTACTGCGCT
OPS-02	CCTCTGACTG

Table 5. Primer sequences used for *Eco*RI/*Mse*I AFLP analysis in Xap and Xapf genetic studies

Name	Type	Sequence (5'-3')
E-A	*Eco*RI Primer+1	AGACTGGTACCAATTCA
E-AA	*Eco*RI Primer+2	GACTGCGTACCAATTCAA
E-AG	*Eco*RI Primer+2	GACTGCGTACCAATTCAG
E-AT	*Eco*RI Primer+2	GACTGCGTACCAATTCAT
E-AAC	*Eco*RI Primer+3	GACTGCGTACCAATTCAAC
E-ACC	*Eco*RI Primer+3	GACTGCGTACCAATTCACC
E-AACA	*Eco*RI Primer+4	GACTGCGTACCAATTCAACA
E-AACC	*Eco*RI Primer+4	GACTGCGTACCAATTCAACC
M-C	*Mse*I Primer+1	GACGATGAGTCCTGAGTAAC
M-CAA	*Mse*I Primer+3	GATGAGTCCTGAGTAACAA
M-CAC	*Mse*I Primer+3	GATGAGTCCTGAGTAACAC
M-CAG	*Mse*I Primer+3	GATGAGTCCTGAGTAACAG
M-CAT	*Mse*I Primer+3	GATGAGTCCTGAGTAACAT
M-CTA	*Mse*I Primer+3	GATGAGTCCTGAGTAACTA
M-CTC	*Mse*I Primer+3	GATGAGTCCTGAGTAACTC
M-CTG	*Mse*I Primer+3	GATGAGTCCTGAGTAACTG
M-CTT	*Mse*I Primer+3	GATGAGTCCTGAGTAACTT

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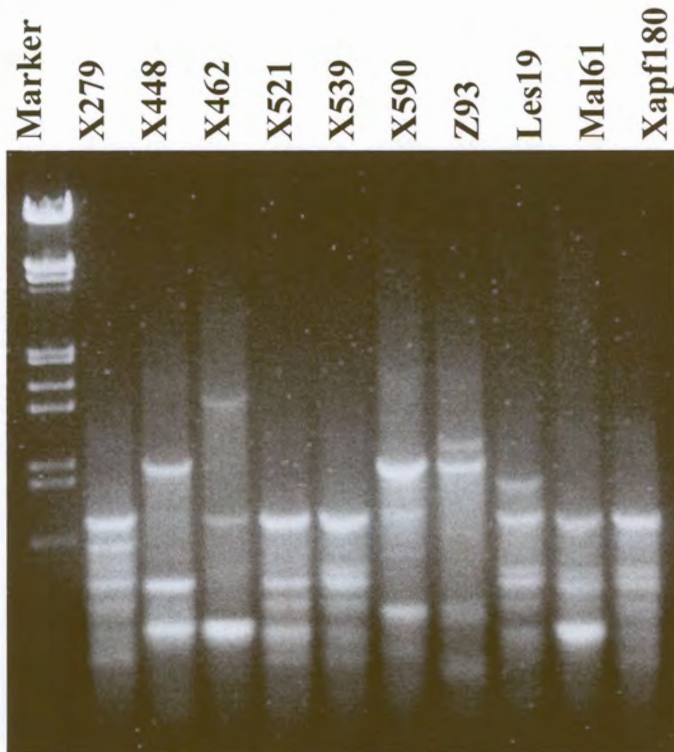

Figure 1. RAPD analysis of 2 Xap (X448 and X590), 7 Xapf (X279, X521, Les 19, Xapf 180, Mal 61, X539 and X462) and 1 non-pathogenic *Xanthomonas* (Z93) isolates

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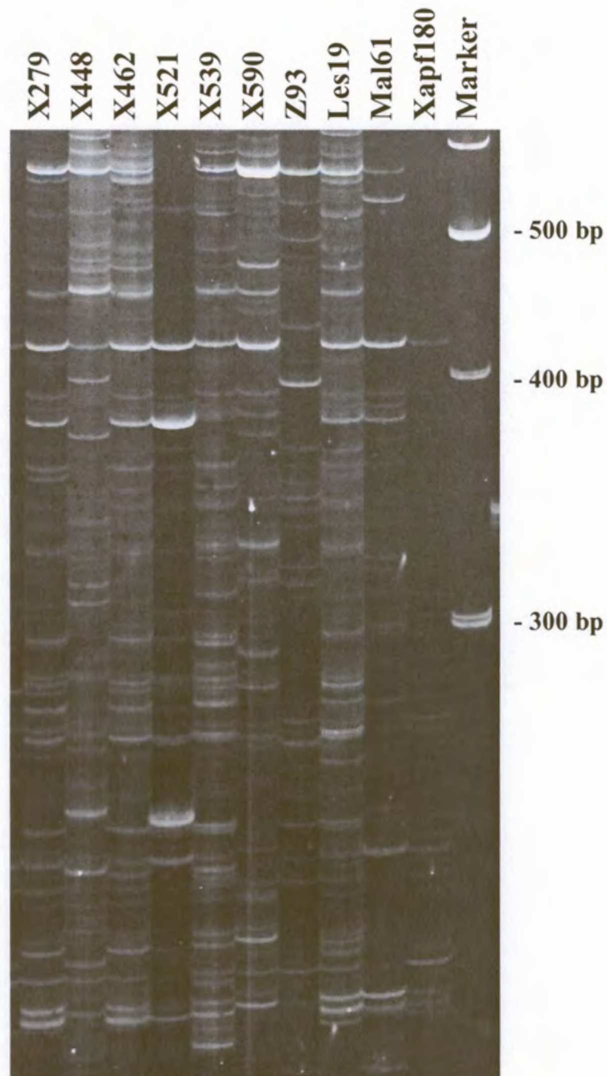

Figure 2. AFLP analysis of 2 Xap(X448 and X590), 7 Xapf (X279, X521, Les 19, Xapf 180, Mal 61, X539 and X462) and 1 non-pathogenic *Xanthomonas* (Z93) isolates

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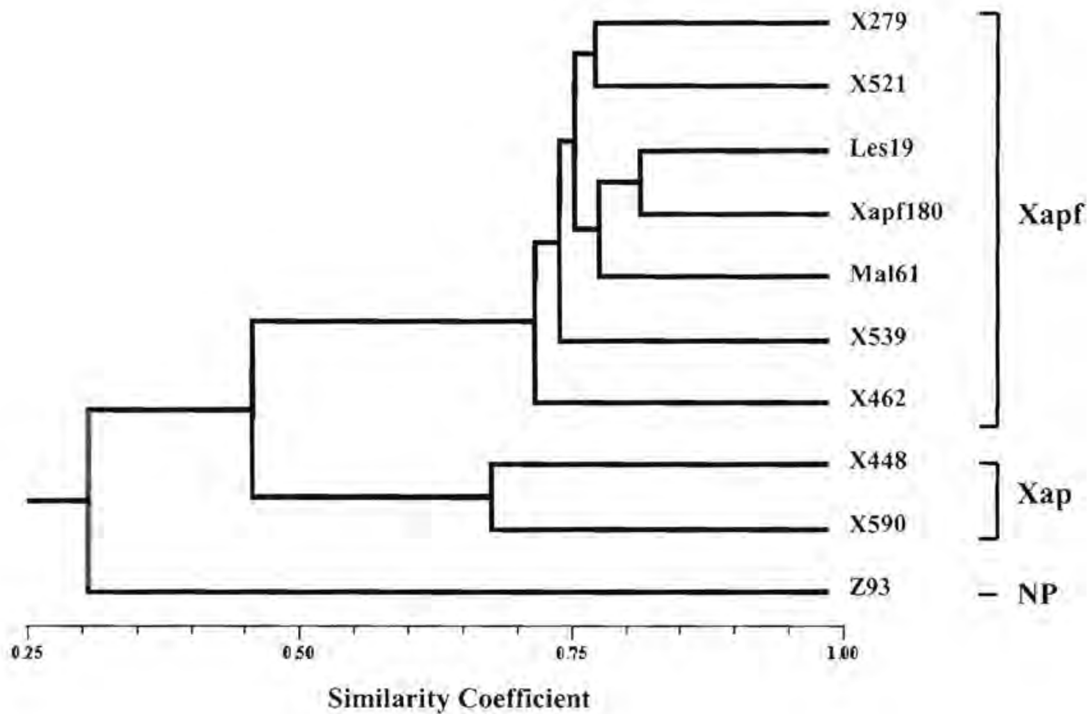

Figure 3. Genetic relationship of 2 Xap, 7 Xapf and 1 non-pathogenic *Xanthomonas* isolates based on combined RAPD and AFLP data