Verticillium wilt of potato in South Africa

by

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Submitted to the Faculty of Natural and Agricultural Sciences

Department of Microbiology and Plant Pathology

In partial fulfilment of the requirements for the degree of

M.Sc. Plant Pathology

UNIVERSITY OF PRETORIA JANUARY 2003

ACKNOWLEDGEMENTS

My sincere appreciation to the following persons and institutions for their contribution to this dissertation:

Prof F.C. Wehner, my supervisor, for his inspiring and able leadership, advice, criticism and help during the preparation of this manuscript.

The ARC-Roodeplaat Vegetable and Ornamental Plant Institute who made this study possible.

The Potato Producers Organisation for granting me the opportunity through their financial support.

My colleagues, in particular William Nkadimeng and the late Lazarus Mothoa, who played a vital role in making this study possible. Also to Dr. Freddie Denner and Annette Swanepoel for always being accessible and providing the means to much needed experience.

My husband Sollie, children and parents for their love and support in the pleasant and unpropitious times.

Finally, my Creator for giving me the strength and courage to complete this study.

CONTENTS

CHAPTER 1
GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) originated in the Andes mountains of South America. It belongs to the Solanaceae, a plant family including various important commercial crops like tomato (*Lycopersicon esculentum* Mill.), peppers (*Capsicum* spp.), brinjal (*Solanum melongena* L.) and tobacco (*Nicotiana tabacum* L.), as well as weeds such as black nightshade (*Solanum nigrum* L.), thorn apple (*Datura stramonium* L.) and bitter apples (*Solanum elaeagnifolium* Cav., *S. rostratum* Dunal, *S. sisymbrifolium* Lam., etc.) (Rowe, 1993; Bromilow, 1995). Potatoes are of major importance in human nutrition, ranking fourth in world consumption after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Rowe, 1993). In South Africa, potatoes are the third most important staple food after maize and wheat, and the main vegetable crop, representing 44 % of the total vegetable production (Anonymous, 2002). According to statistics of Potatoes South Africa (http://www.potatoes.co.za), approximately 1.6 million tonnes of potatoes were produced on 53 786 ha in 2001 in the 14 potato production areas of South Africa. Seed production represented 13 % of the total crop production. On average, 63 % of all potatoes produced in South Africa are sold directly or on fresh produce markets, 17 % is processed and 7 % exported.

Verticillium wilt is a vascular disease occurring in both irrigated and non-irrigated potato production areas throughout the world (Tsror & Nachmias, 1995; Plasencia *et al.*, 1996; Arbogast *et al.*, 1999). Infection causes loss of turgor in leaves followed by chlorosis and necrosis, resulting in premature senescence of the foliage, thereby shortening the growth period (Plasencia *et al.*, 1996; Arbogast *et al.*, 1999). Depending on severity, time of occurrence and season, the number and size of progeny tubers may be substantially reduced, the earlier the onset of senescence, the greater the reduction in yield (Arbogast *et al.*, 1999). Yield losses can be as high as 50 % in susceptible cultivars, and 20-30 % in tolerant cultivars if control is inadequate (Nachmias & Krikun, 1985; Tsror & Nachmias, 1995; Plasencia *et al.*, 1996; Arbogast *et al.*, 1999). The only record of *Verticillium* wilt of potato in South Africa is by Doidge (1950), who ascribed it to infection by *Verticillium albo-atrum* Reinke & Berthier.

CAUSAL AGENT

The genus *Verticillium* (kingdom *Fungi*, form-phylum *Deuteromycota*, form-class *Hyphomycetes*, form-order *Hyphales*, series *Phialosporae*, subseries *Phialogloeosporae*) was erected in 1816 by C.G.D. Nees von Esenbeck according to conidiophore morphology. Conidiophores are usually well-differentiated and erect, septate and verticillately branched over most of their length, bearing whorls of slender flask-shaped divergent phialides with inconspicuous collarettes (Fig. 1.1). Conidia are elliptical, ovate-oblong or spherical, mostly unicellular, hyaline or very slightly coloured (Fig. 1.2), borne in slimy heads, exceptionally in chains (Isaac, 1967; Domsch *et al.*, 1980). Of the *ca.* 40 accepted *Verticillium* species, five are known to be pathogenic to potato, viz. (in decreasing order of virulence) *V. albo-atrum*, *V. dahliae* Kleb., *V. nigrescens* Pethybr., *V. nubilum* Pethybr. and *V. tricorpus* I. Isaac. (Robinson *et al.*, 1957; Smith, 1965; Isaac & Harrison, 1968; Schnathorst, 1981). These five species can be distinguished according to their resting structures, *V. albo-atrum* producing dark resting mycelium, *V. dahliae* discrete microsclerotia (Fig. 1.3), *V. nubilum* large chlamydospores (8.5-17 µm diam.), commonly in chains, *V. nigrescens* small chlamydospores (5.5-8 µm diam.), usually formed singly, and *V. tricorpus* chlamydospores as well as large microsclerotia and orange-yellow prostrate hyphae (Smith, 1965; Isaac *et al.*, 1971; Domsch *et al.*, 1980).

V. albo-atrum and *V. dahliae* are considered to be the more common causal agents of *Verticillium* wilt of potato (Davis, 1985; Rowe, 1985; Sundaram *et al.*, 1991). Since the description of *V. dahliae* in 1913 its relationship with *V. albo-atrum* has been subject to much controversy and, prior to about the mid-1900's, they were often regarded to be one variable species, referred to as *V. albo-atrum* (Van den Ende, 1958). Most recent workers, however, consider them as separate (Rowe, 1985). Besides being morphologically distinct, the two species also differ in physiology. For instance, *V. dahliae* is capable of growing at 30 °C while *V. albo-atrum* is not (Isaac, 1949, 1967). Optimum pH for growth of *V. albo-atrum* is 8.0-8.6 (Isaac, 1967) compared to 5.3-7.2 for *V. dahliae* (Isaac, 1949, 1967). *V. albo-atrum* furthermore is much more sensitive to ultra-violet irradiation than *V. dahliae* (Puhalla, 1973) and grows better on the polyhedric alcohols D-mannitol, D-glucitol and D-ribitol (Vega & Le Tourneau, 1971), and the amino acids L-threonine, L-valine and L-arginine, whereas the opposite is true for L- lysine (Selvaraj, 1975). Suitable media to distinguish the two species include prune extract agar (Talboys, 1960) and dilute soil-extract agar with 0.2 % polygalacturonic acid (Green & Papavizas, 1968). Although having virtually the same GC-content, 59.5 % in *V. albo-atrum* and58.7 % in *V. dahliae* (Domsch *et al.*, 1980), molecular

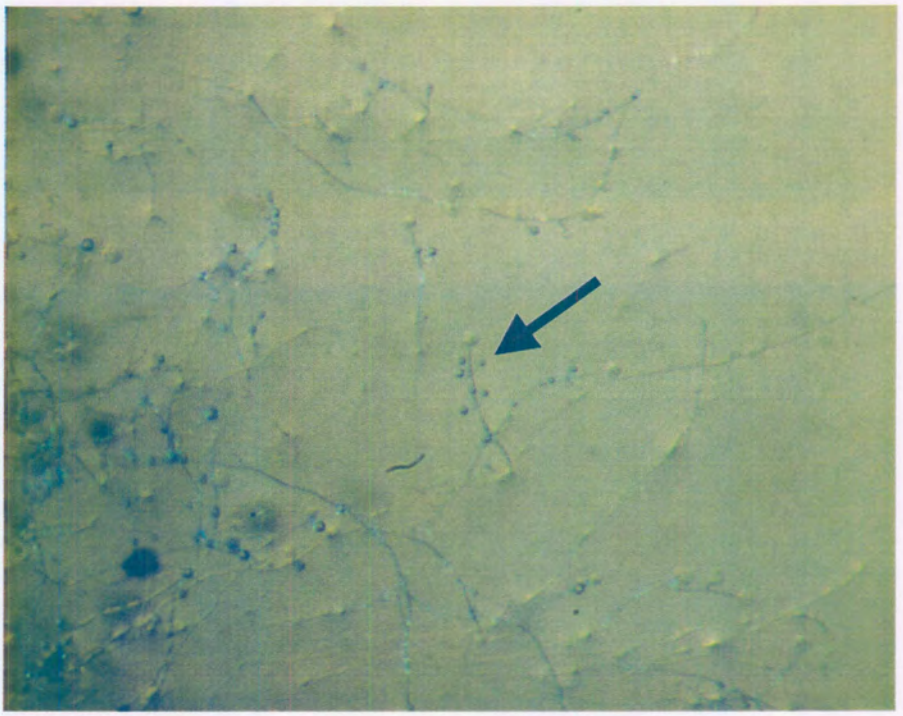

Fig. 1.1. Branched conidiophores of *Verticillium dahliae* with conidia borne terminally in slimy heads on whorls of phialides (arrow) (63 x).

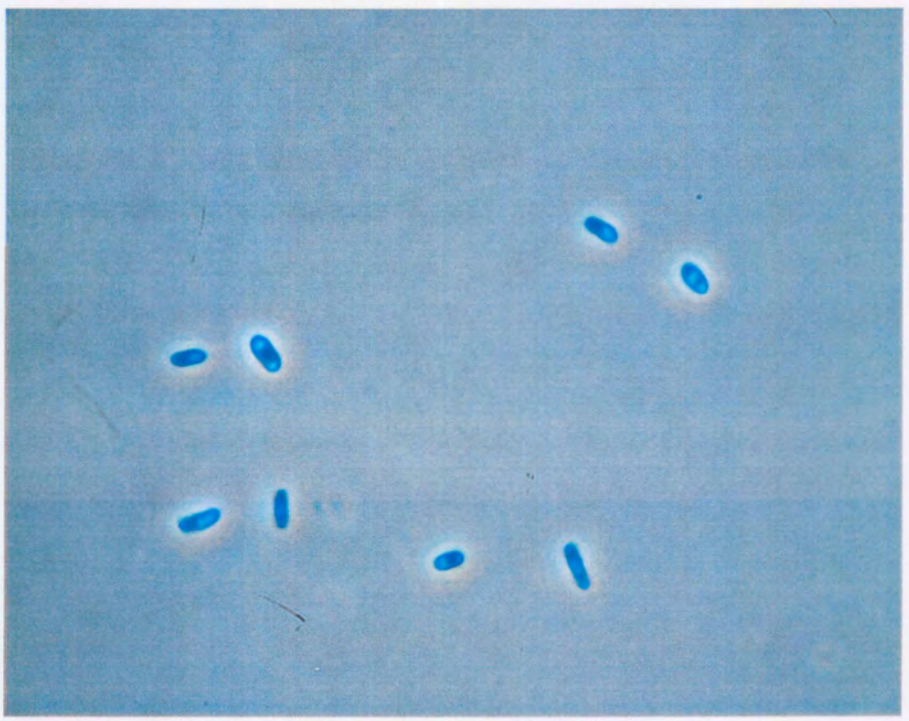

Fig. 1.2. Conidia of *Verticillium dahliae* (400 x).

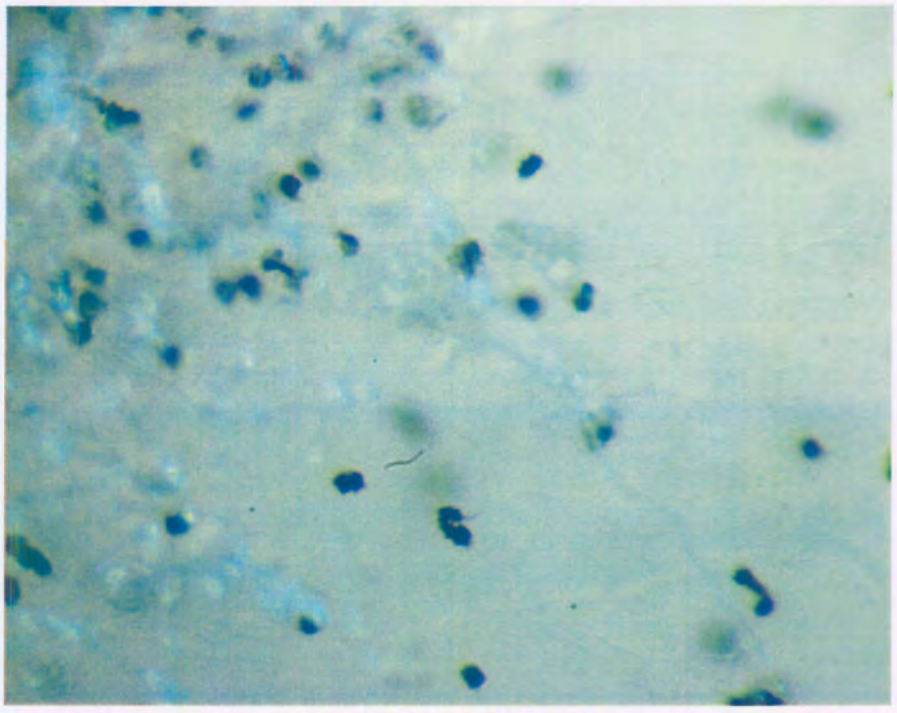

Fig. 1.3. Microsclerotia of *Verticillium dahliae* (63 x).

studies have indicated distinctive species-specific patterns for the two species (Carder & Barbara, 1991; Koike *et al.,* 1995).

In view of the rather northern distribution of *V. albo-atrum* in Europe, the USA and Canada (Devaux & Sackston, 1966; Isaac, 1967; Domsch *et al.,* 1980), contrary to *V. dahliae* which is widely distributed between latitudes 60 °N and 50 °S in temperate and subtropical zones in all continents (Domsch *et al.,* 1980; Harris, 1998; Soesanto, 2000) and due to the results of the survey presented in Chapter 2, the rest of this chapter will focus on *V. dahliae*.

HOST RANGE

V. dahliae has a world-wide distribution, causing vascular wilt diseases in more than 160 plant species, including vegetables such as artichoke (*Cynara cardunculus* L.), brinjal, Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), cabbage (*Brassica oleracea* L. var. *capitata* L.), cucumber (*Cucumis sativus* L.), potato, tomato and sweet pepper (*Capsicum frutescens* L.), fruit and nut crops such as apricot (*Prunus armeniaca* L.), avocado (*Persea americana* Mill.), cherry (*Prunus avium* x *cerasus* L.), vine (*Vitis vinifera* L.), olive (*Olea europaea* L.), pistachio (*Pistacia vera* L.), raspberry (*Rubus*

idaeus L.), strawberry (*Fragaria* x *ananassa* Duch.) and watermelon (*Citrullus lanatus* (Thunb.) Matsumura & Nakai), field crops such as cotton (*Gossypium hirsutum* L.), groundnut (*Arachis hypogaea* L.), hops (*Humulus lupulus* L.) and lucerne (*Medicago sativa* L.), various forest and shade trees, and woody and herbaceous ornamentals such as *Antirrhinum* and rose (*Rosa* spp.) (Campbell & Griffiths, 1973; Nachmias *et al.*, 1982; Rowe, 1985; Subbarao *et al.*, 1995; Subbarao & Hubbard, 1996; Hiemstra, 1998; Visser, 1999; Soesanto, 2000). Weeds such as black nightshade, shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) and species in the genera *Chenopodium, Lamium, Medicago* and *Tagetes* are reported to be hosts of *V. dahliae* as well (Vargas-Machuca *et al.*, 1987).

A study by Subbarao *et al.* (1995) indicated that *V. dahliae* lacks host specificity, as isolates from non-cruciferous crops were as virulent on cauliflower as cauliflower isolates, and vice versa. In South Africa, *V. dahliae* isolates from cotton, potato and avocado all caused typical *Verticillium* wilt symptoms in tomato seedlings and were successfully re-isolated from the seedlings (Visser, 1999). However, there can be a certain degree of host specialisation in that isolates often are more virulent on the host from which they have originally been isolated than on other hosts (Bhat & Subbarao, 1999; Visser, 1999).

V. dahliae comprises three vegetative compatibility groups (VCG's) that each has been divided into two subgroups, namely VCG1A, VCG1B, VCG2A, VCG2B, VCG4A, and VCG4B (Katan, 2000). These VCG's exhibit some specialisation with respect to host range, aggressiveness and distribution. For example, VCG4A is most commonly encountered on solanaceous crops in Europe and America (Soesanto, 2000). Although VCG4 has been recovered from various host plants, extensive surveys indicated that potato is its preferred host. In several studies, isolates belonging to VCG4 collectively exhibited significantly higher virulence on potato than other VCG's. In further comparisons within VCG4, isolates of VCG4A were collectively more virulent than those of VCG4B, the latter not differing significantly from VCG2 isolates (Katan, 2000). The virulence of a particular isolate of *V. dahliae* obviously also depends on the susceptibility of the host cultivar (Soesanto, 2000). Furthermore, in potato, VCG4A but not VCG4B, was shown to interact with the root-lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven, thus aggravating the early-dying syndrome (Katan, 2000).

SYMPTOMOLOGY AND EPIDEMIOLOGY

V. dahliae may enter directly into the stems of potato plants growing from infected tubers, or may penetrate the roots of plants in infested soil (Robinson *et al.*, 1957). Growth of new mycelium appears to originate from old hyaline hyphae associated with microsclerotia, as germination of the dark, thick-walled microsclerotial cells themselves has not been observed (Schnathorst, 1962). Microsclerotial propagules are stimulated to germinate in soil by root exudates, and were found to germinate and sporulate each time after up to nine consecutive cycles of air-drying and re-wetting (Farley *et al.*, 1971). When in contact with the host, hyphae penetrate the roots directly and progress to the vascular tissue. As colonisation of the xylem proceeds the vessels become plugged by hyphae, and a light brown discoloration of vascular tissue in the stems (Fig. 1.4) and tubers (Fig. 1.5) is a common feature of vascular invasion (Robinson *et al.*, 1957; Isaac & Harrison, 1968; Schnathorst, 1981; Rowe, 1985; Bowden & Rouse, 1991; Xiao & Subbarao, 1998). *V. dahliae* secretes pectinolytic enzymes that destroy the middle lamellae of the xylem parenchyma and degrade pectic compounds in the walls of vessels and tracheids. Parenchyma cells adjacent to the infected xylem vessels become discoloured and filled with a gum-like substance. Infected xylem vessels may become clogged by the secretion of gum-like substances by the parenchyma cells and by masses of fungal hyphae. This, in combination with production of wilt toxins by the pathogen, result in reduced respiration, impaired photosynthesis and, finally, wilting (Soesanto, 2000). Endo-polygalacturonase is the main wilt toxin (Wang & Keen, 1970; Selvaraj, 1973), but extracellular lipo-polysaccharides have also been implicated (Keen *et al.*, 1970). Three fractions of endo- and one of exo-1,4-ß-glucanase are produced (Russel, 1975). Production of catalase, cellulases and ß-fructofuranosidase is positively correlated with the degree of pathogenicity on cotton (Domsch *et al.*, 1980). *V. dahliae* also produces a growth substance with gibberellin-like properties (Aube & Sackston, 1965).

Following systemic colonisation, symptom development occurs as a result of vascular dysfunction, but symptoms are difficult to distinguish from normal senescence. Advanced symptoms do not usually occur until after flowering and may involve the decline of isolated plants or, in severe cases, early maturing of an entire crop. Stomatal resistance of leaves on infected plants increases, resulting in reduced concentrations of CO_2 within leaves and consequent decrease in net photosynthesis, hence explaining the characteristic yellowing symptoms (chlorosis) (Fig. 1.6) (Rowe, 1985; Bowden & Rouse, 1991; Xiao & Subbarao, 1998). All the leaflets along one side of the petiole, and occasionally the terminal leaflet of the

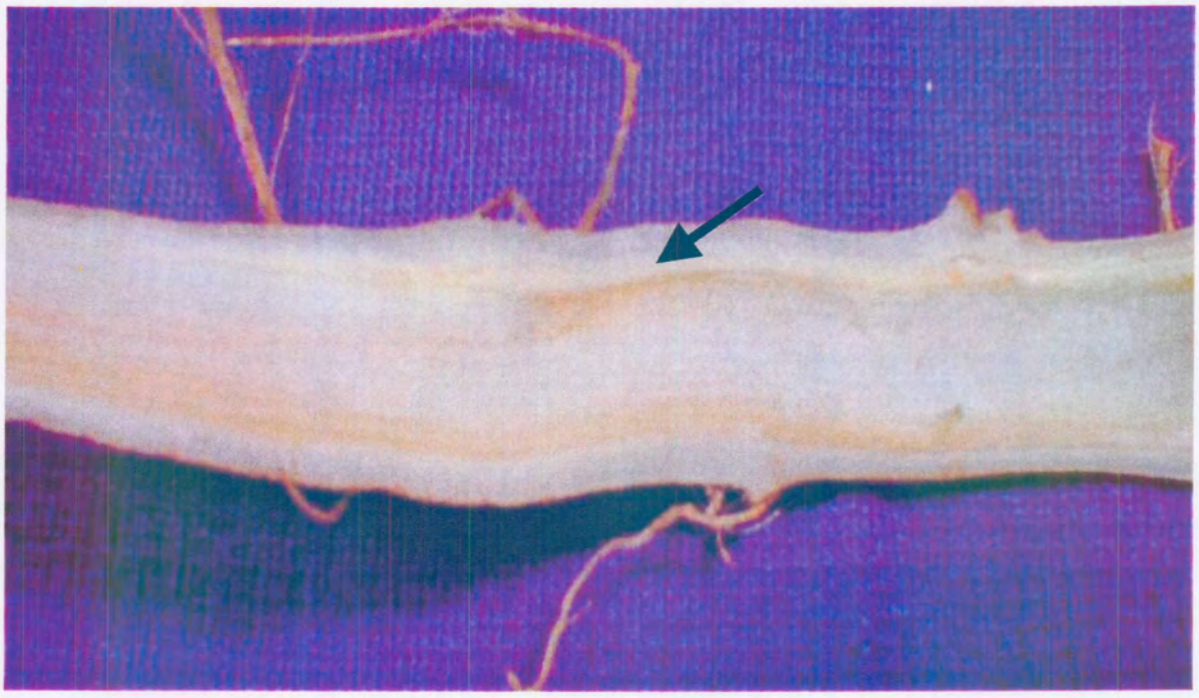

Fig. 1.4. Light brown discoloration of vascular tissue of a potato stem infected with *Verticillium dahliae*.

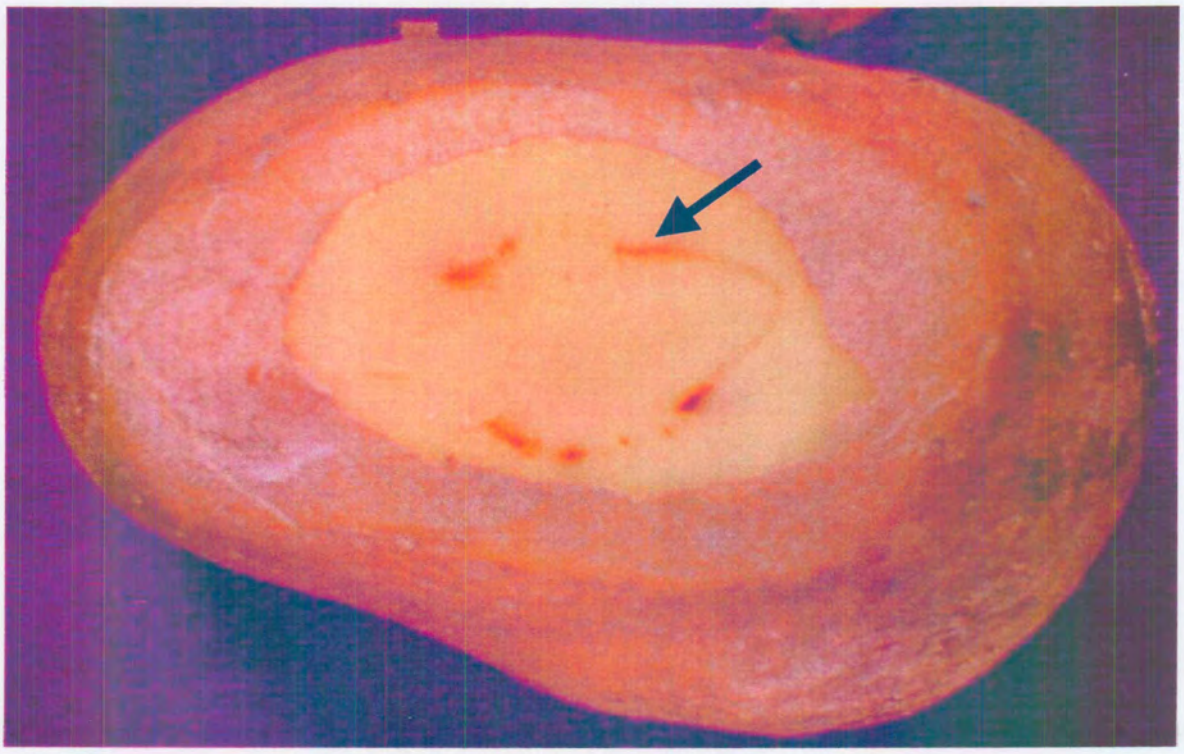

Fig. 1.5. Light brown discoloration of vascular tissue of a potato tuber infected with *Verticillium dahliae*.

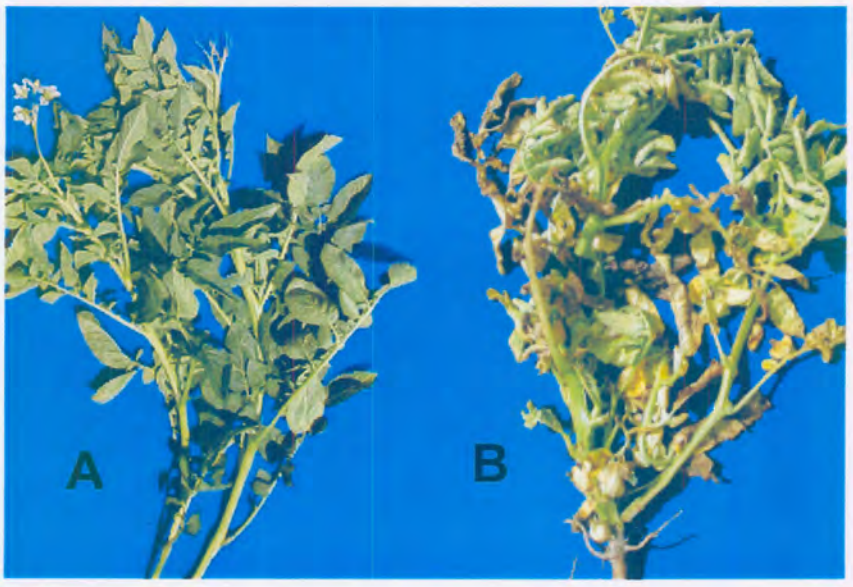

Fig. 1.6. Characteristic yellowing and wilting of potato leaves due to *Verticillium* wilt.
A) Healthy plant
B) Diseased plant

lower leaves, become pale and chlorotic. This is followed by necrosis, which progresses from the apices and margins inwards. The necrotic leaves usually drop prematurely, leaving only a few shrivelled specimens remaining on the plant, and the lower portion of the stem completely defoliated. Leaf yellowing and death proceed up the stems, which usually remains erect (Isaac & Harrison, 1968; Rowe, 1985). Water stress caused by decreasing hydrolic conductance of the xylem induces the typical wilting symptoms (Bowden & Rouse, 1991; Xiao & Subbarao, 1998). Uneven epinasty and wilting of the leaves are usually the first permanent symptoms. The lower leaves rapidly lose turgor and show an irreversible wilt, which gradually progresses to involve the whole plant (Robinson *et al.*, 1957; Isaac & Harrison, 1968; Rowe, 1985). Conidia that are formed are transported with the xylem fluid and germinate to form new infections at remote sites such as stolons and newly developing tubers. As the host dies, the entire plant is colonised and resting structures are formed within the necrotic tissue to give the stems a distinctive grey colour and turn the roots and stolons black. The resting structures are eventually incorporated into the soil with the plant debris, increasing the level of inoculum for successive crops (Robinson *et al.*, 1957; Schnathorst, 1981; Rowe, 1985; Bowden & Rouse, 1991). The principal source of inoculum of *V. dahliae* is microsclerotia, which can survive in soil for up to 13 years in the absence of a host (Schnathorst, 1981).

Disease development and symptom expression are affected by climate, including temperature and day length, as well as agronomic practices such as irrigation and rate of nitrogen application (Nachmias *et al.*, 1990; Arbogast *et al.*, 1999). When air temperatures are in the optimal range for colonisation of vascular tissue by *V. dahliae*, disease can be severe (Arbogast *et al.*, 1999).

Potatoes grow optimally within a temperature range of 18-20 °C, whereas the optimal range for growth of *V. dahliae* is 21-27 °C. In accordance with these optima, disease severity in potatoes infected with *V. dahliae* tends to increase as the mean air temperature increases from 20 to 28 °C (Powelson & Rowe, 1993). *Verticillium* wilt is more severe during a wet season and/or excessive irrigation than in a dry season with moderate or insufficient irrigation (Cappaert *et al.*, 1992; Powelson & Rowe, 1993), because severity of the disease is related to the level of soil water (Cappaert *et al.*, 1994; Arbogast *et al.*, 1999). Excessive irrigation may have an effect on movement of conidia of *V. dahliae* from sporulating microsclerotia to the root surface and can increase the movement of conidia in the vascular tissue, which could affect the rate of vascular colonisation and wilt development (Powelson & Rowe, 1993; Xiao *et al.*, 1998). N availability is commonly associated with *Verticillium* wilt. Colonisation of plant tissue by *V. dahliae* decreases with an increase in availability of N. As the availability of N approaches the optimal for yield, disease severity is less, i.e. the relationship is inversely related (Davis & Everson, 1986). Excessive nitrogen rates induce excessive vegetative growth, leaving the plant with thin cell walls. This in turn results in a weak plant, with lower resistance to wilt pathogens. Adequate ammonium levels to stimulate good plant growth, without promoting excessive growth, enhance resistance to *Verticillium* wilt (Uys, 1996).

DETECTION METHODS

Contamination of uninfested fields with *V. dahliae* can occur by wind or mechanical movement of soil particles containing viable propagules, but the primary mode of introduction is by means of infected seed stock. Because *V. dahliae* can infect potato tubers through stolons and persist as dormant mycelium in the vascular tissue (Rowe, 1985; Nagtzaam *et al.*, 1997) it is important to have available a fast, specific, sensitive and inexpensive method to detect the pathogen in plant material and ensure that only disease-free seed is used. Plasencia *et al.* (1996) developed a monoclonal antibody-based immunoassay for detecting *V. dahliae* in vascular tissue. Whereas conventional culture plating quantifies fungal propagules, the immunoassay detects soluble protein as indicator of fungal biomass, and proved to reliably detect vascular colonisation by *V. dahliae* in potato (Plasencia & Banttari, 1997). The immunoassay is also

quicker, since results can be obtained in 2 to 4 days, and allows for retesting of the sample. The capacity to process a large number of samples widens the potential application of the immunoassay to epidemiological studies requiring information on disease incidence and/or severity. The immunoassay does not provide an improvement in sensitivity or reliability over the culture plate method but offers the possibility of increasing sample size at no increase in cost. The availability of *V. dahliae*–specific PCR (Polymerase Chain Reaction) primers may aid in more rapid and specific detection of the pathogen directly in plant and/or soil samples (Messner *et al.,* 1996; Plasencia *et al.*, 1996; Perez-Artes *et al.*, 2000). Primer sets were designed based on small differences in the genomic sequences identified in the internal spacer regions of ribosomal genes. However, for detection and quantification in a large number of plants, the immunoassay offers advantages in terms of cost (Plasencia *et al.*, 1996).

CONTROL

Reduction in soilborne microsclerotia within an acceptable period usually is accomplished by a combination of chemical and cultural methods (Davis *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999). The disease can be effectively controlled by a number of fumigants, e.g. chloropicrin, methyl bromide and metham-sodium, but their usage is limited because they are expensive, hazardous and laborious to apply (Davis, 1985; Davis & Everson, 1986; Davis & Sorensen, 1986; Rowe *et al.*, 1987; Nachmias *et al.*, 1990; Powelson & Rowe, 1993). Also, the pending withdrawal of methyl bromide will reduce the availability of effective fumigants, and potato growers therefore have to adopt alternative strategies for managing *Verticillium* wilt (Davis & Sorensen, 1986; Davis *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999; Blok *et al.*, 2000). Interest in non-chemical approaches to the management of soilborne diseases has been rekindled with the recent emphasis on sustainable agriculture (Subbarao *et al.*, 1999). The disease may be either suppressed or controlled by a variety of alternative procedures, including resistant cultivars (Davis, 1985; Rowe *et al.*, 1987; Nachmias *et al.*, 1990; Powelson & Rowe, 1993; Tsor & Nachmias, 1995; Davis *et al.*, 1996), long-term crop rotation (Joaquim *et al.*, 1988; Easton *et al.*, 1992; Powelson & Rowe, 1993; Cappaert *et al.*, 1994; Subbarao *et al.*, 1995; Subbarao & Hubbard, 1996; Xiao & Subbarao, 1998; Xiao *et al.*, 1998; Arbogast *et al.,* 1999) and cultural practices that involve incorporation of organic amendments (Easton *et al.*, 1992; Powelson & Rowe, 1993; Subbarao *et al.*, 1995; Davis *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Arbogast *et al.,* 1999; Subbarao *et*

al., 1999; Blok *et al.*, 2000).

V. dahliae does not necessarily have to be introduced by seed stocks into new lands, but in many cases may occur saprophytically on roots of natural vegetation. In these cases, serious outbreaks could result from increases in the populations already present in the soil due to intensive cultivation of a highly susceptible host such as potato. Because of its wide host range, the fungus can survive on roots and plant debris of many crop and weed species as well as on those of non-host species e.g. maize (Fig. 1.7) (Green, 1980; Rowe, 1985; Rowe *et al.*, 1987; Joaquim *et al.*, 1988; Easton *et al.*, 1992; Powelson & Rowe, 1993; Nagtzaam *et al.*, 1997; Visser, 1999). Considering the prolonged survival of microsclerotia, a key to managing *Verticillium* wilt is to reduce the number of microsclerotia in soil to levels too low to cause disease in susceptible crops (Rowe *et al.*, 1987; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999; Blok *et al.*, 2000). Threshold levels for disease vary from 0.6 to more than 46 colony-forming units g^{-1} soil (Mol *et al.*, 1996). The reasons for this wide range of threshold levels are that *V. dahliae* is a weak competitor in the rhizosphere, and that disease development and plant growth are influenced by temperature and other environmental factors such as availability of water and mineral nutrients. The infection rate, therefore, not only depends on the inoculum density but also on interactions with soil microorganisms and effects of abiotic factors.

Developing genetically-stable resistant or tolerant cultivars is considered to be the most efficient, economical and environmentally sound approach to control *Verticillium* wilt in potato (Rowe *et al.*, 1987; Nachmias *et al.*, 1990; Powelson & Rowe, 1993; Tsror & Nachmias, 1995; Plasencia & Banttari, 1997). Plant resistance to *Verticillium* wilt is defined as the inability of the pathogen to penetrate the roots, interference with host tissue colonisation, inactivation of toxic elements, or suppression of sporulation (Tsror & Nachmias, 1995). Certain potato varieties show delayed or reduced colonisation after penetration by *V. dahliae* but do not suffer from severe wilt symptoms or a reduction in the marketable yield of tubers, and are therefore considered tolerant (Tsror & Nachmias, 1995; Plasencia & Banttari, 1997). Although tolerance is acceptable in some circumstances, it is not as desirable as resistance to infection because, under successive cropping, the pathogen will eventually build up in the soil to a level at which tolerance is no longer effective in preventing yield losses (Plasencia & Banttari, 1997).

Fig. 1.7. Microsclerotia of *Verticillium dahliae* (arrow) occurring saprophytically on a maize stem (20 x).

Crop rotation generally improves soil structure, moisture absorption and retention, fertility and other variables that affect yield. Maize, barley (*Hordeum vulgare* L.), bluegrass (*Poa* spp.), carrot (*Daucus carota* L.), mung bean (*Vigna radiata* (L.) R. Wilcz), wheat (*Triticum aestivum*. L.), grain sorghum (*Sorghum bicolor* (L.) Moench), and sugar beet (*Beta vulgaris* L.) are potential rotation crops considered nonhosts of *V. dahliae* (Easton *et al.*, 1992). However, despite the agronomic advantages of crop rotation, there are conflicting reports on the effectiveness of the practice in controlling *Verticillium* wilt caused by *V. dahliae*. After an eight-year cycle of rotating potatoes with maize or barley, Davis & McDole (1979) could find no significant reductions in *V. dahliae* populations, whereas Easton *et al.* (1975) reported total eradication of *V. dahliae* populations from soil cropped to potato after one year of wheat. Short crop rotations rarely are effective in eradicating *V. dahliae*, because of the slow attrition rate of microsclerotia in soil, inoculum density level well above economic threshold at the onset of rotation, and wide host range of the pathogen. Furthermore, germinating microsclerotia may colonise roots of nonsusceptible weeds or crops such as

oats (*Avena sativa* L.), wheat, barley and grain sorghum at low levels, thereby maintaining inoculum densities sufficient to infect susceptible hosts. The release of microsclerotia from incorporated plant residues containing microsclerotia are the major source of inoculum for the following season's crop, especially if the release is higher than the decrease in microsclerotia due to mortality (Harrison & Isaac, 1969; Joaquim *et al.*, 1988; Easton *et al.*, 1992; Mol *et al.*, 1996; Xiao & Subbarao, 1998; Xiao *et al.*, 1998).

For crop rotation to be effective with a persistent pathogen such as *V. dahliae*, the crop selected for rotation with the susceptible host should (a) result in a reduction in soilborne microsclerotia and a concomitant reduction in wilting of the susceptible crop and (b) be compatible with current production practices (Xiao *et al.*, 1998). It is important to remember that *V. dahliae* cannot be controlled by short-term rotations with any of the potential rotation crop species. Furthermore, crop rotation is only part of an integrated management system for managing *V. dahliae* (Mol *et al.*, 1996).

An area that is currently being actively researched is biofumigation, i.e. the use of brassicaceous crops to control disease (Subbarao & Hubbard, 1996; Brown & Morra, 1997). Disease control is ascribed to the chemical breakdown of glucosinolates, sulphur-containing compounds, responsible for the inherently pungent odour of brassicaceous plants (Mayton *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999; Vaughn, 1999). They are a group of secondary sulphur compounds composed of a thioglucose group, a variable carbon side chain (R-group) and a sulphonated oxide (Mayton *et al.*, 1996; Smolinska *et al.*, 1997; Vaughn, 1999). Glucosinolates are named according to their R-group (Mayton *et al.*, 1996). During the decomposition of brassica residues, glucosinolates, contained in vacuoles, are hydrolysed by the enzyme myrosinase (α-thioglucosidase glucohydrolase), which is present in the cell walls, endoplasmic reticulum, Golgi vesicles and mitochondria, to various biologically active hydrolysis products such as isothiocyanates, thiocyanates, nitriles, epinitriles and sulphides. Some of the hydrolytic breakdown products have either fungistatic or fungicidal, antimicrobial and insecticidal properties (Mayton *et al.*, 1996; Subbarao & Hubbard, 1996; Smolinska *et al.*, 1997; Subbarao *et al.*, 1999; Vaughn, 1999). The end-product of the hydrolytic reaction is determined by the R-group of the glucosinolate and the physical and chemical conditions under which hydrolysis takes place. Allyl glucosinolate is generally converted to allyl isothiocyanate (AITC) at a pH of 4.0 or greater. AITC, a volatile compound, is as toxic to fungi as methyl isothiocyanate, the active

ingredient in commercial soil fumigants such as dazomet and metham-sodium (Tomlin, 1994; Mayton *et al.*, 1996; Smolinska *et al.*, 1997). AITC breaks the disulphide bond of cystine in proteins and glutathione through oxidative cleavage/scission (Vaughn, 1999). Allyl isothiocyanate production in brassicaceous crops increases with increasing levels of sulphur nutrition in soil (Subbarao & Hubbard, 1996). Approximately 100 different glucosinolates have been identified in plant tissue from at least 11 different plant families, principally the Brassicaceae, Capparidaceae and Resedaceae (Mayton *et al.*, 1996; Vaughn, 1999). As many as 15 different glucosinolates can be produced by a single species, and concentrations of individual glucosinolates vary within different organs of the same plant and within populations of the same species (Vaughn, 1999). The concentration of glucosinolates is highest in actively growing tissues and declines as the plant ages (Matthiessen *et al.*, 2000). Thus, varying types and amounts of glucosinolates within the brassica species determine the level of plant pathogen suppression (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999). Control of *V. dahliae* in cauliflower has been achieved through soil incorporation of broccoli (*B. oleracea* L. var. *italica* Plenck) residues (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999).

OBJECTIVES OF THE PRESENT STUDY

Following the first report of *Verticillium* wilt of potato in South Africa from an undisclosed locality in the former Cape Province, and despite the belief of Doidge *et al.* (1953) that the disease could be more prevalent, no new cases were confirmed until 1989. Since then, however, *Verticillium* wilt has increased to such an extent that it now is of major concern to the potato industry in the country (Millard, 1999). The following aspects of the disease have been identified as of high priority for further study in South Africa:

1. Actual incidence of the disease and identity of the causal species in the various potato production areas.
2. Resistance or tolerance of local potato cultivars.
3. An environmentally-compatible control strategy.

Progress made in the above regard is presented in the chapters that follow.

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CHAPTER 2

OCCURRENCE AND DISTRIBUTION OF *VERTICILLIUM* SPECIES ON POTATO IN SOUTH AFRICA

ABSTRACT

Verticillium species were isolated from 146 samples of symptomatic potato plant material received between 1995 and 2000 from 13 of the 14 potato production areas in South Africa. Of ninety-three isolates that were obtained, 60 % were identified as *Verticillium dahliae* and 8 % as *V. nigrescens*. *V. dahliae* was present in nine of the regions and *V. nigrescens* in seven. Unidentified *Verticillium* species were isolated from six of the regions. Both *V. dahliae* and *V. nigrescens* were pathogenic to potato in artificial infection studies, with *V. dahliae* the more virulent of the two species.

INTRODUCTION

Verticillium wilt is a common vascular disease limiting production of potato (*Solanum tuberosum* L.) in both irrigated and non-irrigated cultivation systems throughout the world (Davis & Sorensen, 1986; Tsror & Nachmias, 1995; Plasencia *et al.*, 1996; Xiao & Subbarao, 1998; Arbogast *et al.*, 1999). Five species of *Verticillium* are associated with the disease, viz. *V. albo-atrum* Reinke & Berthier, *V. dahliae* Kleb., *V. nigrescens* Pethybr., *V. nubilum* Pethybr. and *V. tricorpus* I. Isaac (Robinson *et al.*, 1957; Smith, 1965; Isaac & Harrison, 1968; Schnathorst, 1981). Of these, only *V. albo-atrum* has been reported on potato in South Africa, and only from one locality in the former Cape Province (Doidge, 1950). Doidge *et al.* (1953) subsequently indicated that *Verticillium* wilt may be more prevalent, but no new cases of the disease were confirmed in the country for almost 40 years. This could have been due to the characteristic symptoms of *Verticillium* wilt, unilateral chlorosis and necrosis, being obscured by normal senescence symptoms (Isaac & Harrison, 1968). Early maturing caused by *Verticillium* is often also confused with poor nutrition, insufficient irrigation or rainfall, herbicide damage or other diseases, particularly *Fusarium* wilt caused by *Fusarium oxysporum* Schltdl. em. W.C. Snyder & H.N. Hansen f.sp. *tuberosi* W.C. Snyder & H.N. Hansen (Krikun & Orion, 1979).

Since 1989, early senescence not ascribable to any physiological disorder or infection by *Fusarium* has increasingly been observed in several of the potato-growing areas in South Africa, implicating infection by *Verticillium* as the cause (unpublished data). The purpose of the present study was to (i) determine the occurrence and distribution of *Verticillium* spp. in the potato-production areas of South Africa, (ii) identify the *Verticillium* isolates to species level, and (iii) confirm the pathogenicity and virulence of the isolates on potato.

MATERIALS AND METHODS

Isolation and identification

A total of 146 samples of potato plant material received between January 1995 and December 2000 at the ARC-Roodeplaat Potato Diagnostic Service, showing typical symptoms of wilting, yellowing and vascular discoloration, were screened for the presence of *Verticillium*. Stems were washed in running water for 5 minutes, immersed in 1 % sodium hypochlorite for 2 minutes, rinsed in sterile water for 30 seconds, and left to dry at room temperature. Stems were cut longitudinally and five sections of vascular tissue *ca*. 10 x 2 mm in size, from each half of each stem were plated on potato-dextrose agar (PDA) supplemented with 100 mg streptomycin sulphate in 10 ml ethanol l^{-1}. Plates were incubated at 25 °C for 3-5 days and colonies resembling those of *Verticillium* were isolated on PDA + streptomycin. The same procedure was followed with tuber material, except that a thin layer of the stem-end periderm was removed aseptically and isolations made from the tissue underneath.

Isolates were transferred to corn meal agar plates, incubated at 25 °C in the dark for 4 weeks, and the *Verticillium* species identified. Identifications were verified by I.H. Rong of the ARC-Plant Protection Research Institute, Pretoria. After identification the isolates were stored in glycerol at -70 °C.

Pathogenicity testing

Potato (cv. BP1) minitubers were planted to 15-cm-diameter pots containing a 3:1 (v/v) mixture of tyndallised (105 °C for 30 minutes on three consecutive days) sandy soil (7 % clay) and vermiculite. Pots with tubers were maintained in a greenhouse at 25±2 °C. Inoculum was prepared of 37 *V. dahliae* isolates on V8 juice-supplemented vermiculite as described by Denner (1997). Microsclerotia on the vermiculite were enumerated according to the method of Harris *et al.* (1993) and incorporated into the soil:vermiculite mixture at 10 microsclerotia g^{-1} at planting, using 10 pots per isolate. Inoculum of *V. nigrescens* comprised a conidial suspension

containing 10^6 conidia ml^{-1}. Ten millilitres of the conidial suspension of each of four isolates were added to each of 10 pots, 6 weeks after planting. Ten pots without *V. dahliae* or *V. nigrescens* served as control. Fertiliser (1 g 2:3:2 (22) N:P:K) was applied at planting to each pot. Plants were irrigated three times a day for 2 minutes by means of an automated micro-irrigation system.

Plants were examined fortnightly from 8 weeks after planting for the presence of wilting. Stems were divided 12 weeks after planting into three equal sections *ca.* 30 cm long, and a class value assigned to each plant according to a 5-point scale adapted from Robinson *et al.* (1957) and Isaac & Harrison (1968):

1 = no wilting or yellowing
2 = wilting and yellowing in one third of the stem
3 = wilting and yellowing in two thirds of the stem
4 = total wilting and yellowing
5 = whole plant dead

To confirm Koch's postulates, stem isolations were made from plants, 12 weeks after planting, on PDA + streptomycin. Each isolate was classified into a wilt reaction category based on the modified index of Corsini *et al.* (1988):

{(presence of wilt symptoms, 0 or 1) x (wilt severity, 1-5)} + (re-isolation of pathogen, 0 or 1)

Based on the index, isolates were rated as:

≤2.2	= not pathogenic
2.3-4.0	= virulent
≥4.1	= highly virulent

A two-way analysis of variance (ANOVA) using the statistical program GENSTAT (2000) was performed to test for differences in disease index between treatments (control + *V. dahliae* isolates and control + *V. nigrescens* isolates, respectively). Data were acceptably normal with homogeneous treatment variances. Treatment means were separated according to Fishers' protected *t*-test least significant difference at 1 % level of significance if the F-probability from the ANOVA was significant at 1 %.

RESULTS

A total of 93 *Verticillium* isolates were collected from the 146 potato samples (mainly from the cultivars BP1, Up-to-date and Buffelspoort) received from 13 of the 14 potato growing areas, North-eastern Cape being the only region not submitting any specimen (Fig. 2.1). *Fusarium* spp. were also frequently isolated from the samples. Fifty-six of the isolates were identified as *V. dahliae* and seven as *V. nigrescens*. The identity of 30 of the isolates could not be established. More than half of the *V. dahliae* and unidentified *Verticillium* isolates were from the Sandveld. *V. dahliae* was also isolated from the Eastern Cape, Limpopo Province, Ceres, South-western Cape, KwaZulu-Natal, Mpumalanga, Southern Cape and Northwest. Samples from the Sandveld and Eastern Cape, the latter yielding a relatively high number of *V. dahliae* isolates, did not contain *V. nigrescens*. Samples from Limpopo Province, South-western Cape, Mpumalanga, Southern Cape, Western Free State, Eastern Free State and Northern Cape provided one isolate of *V. nigrescens* per region.

Koch's postulates were confirmed for all the *V. dahliae* and *V. nigrescens* isolates tested. The four *V. nigrescens* isolates included were classified as virulent (Table 2.1). Of the 37 *V. dahliae* isolates screened, eight were virulent and 29 highly virulent (Table 2.2).

DISCUSSION

Results of this investigation implicate *V. dahliae* as the main cause of *Verticillium* wilt of potato in South Africa and showed that *V. albo-atrum*, previously reported by Doidge (1950), is absent. The dominance of *V. dahliae* is not surprising as this species is widely distributed in temperate and subtropical zones between 60 °N and 50 °S in all continents, whereas *V. albo-atrum*, with its preference for cooler climates, has a more northern distribution in Europe, the USA and Canada (Devaux & Sackston, 1966; Isaac, 1967; Domsch *et al.*, 1980; Harris, 1998; Soesanto, 2000). That the environment in South Africa favours *V. dahliae* rather than *V. albo-atrum* is evident from a recent compilation of phytopathogenic fungi in the country (Crous *et al.*, 2000) in which the latter species is listed only on cucumber (*Cucumis sativus* L.), besides potato, whereas 11 plant species in six families are entered as hosts for *V. dahliae*. It is likely that Doidge (1950), when describing *V. albo-atrum* on potato, conformed to the taxonomic dispensation of that time (Van den Ende, 1958) and considered *V. dahliae* as conspecific with *V. albo-atrum*, as there is no reference to *V. dahliae* on any plant host in her monumental work.

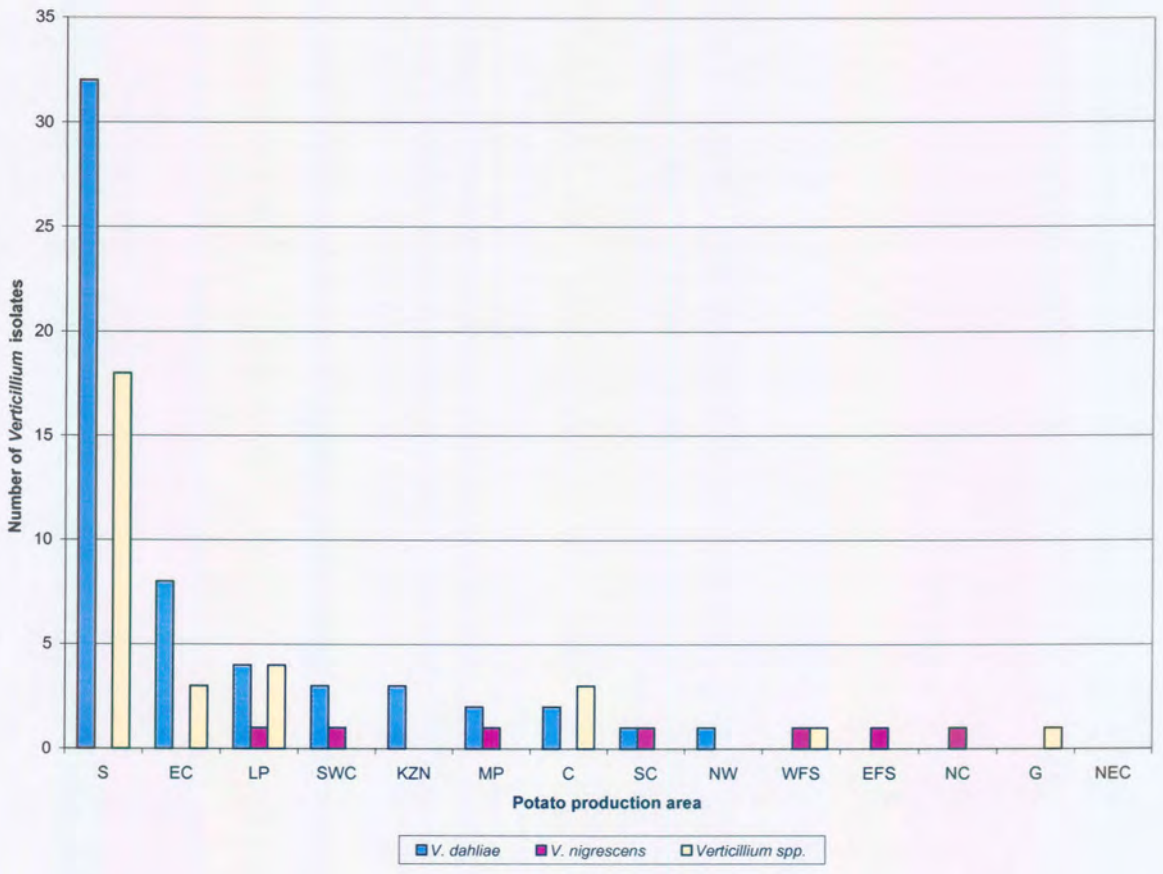

Fig. 2.1. Occurrence and distribution of *Verticillium* spp. isolates in South African potato production areas during the period 1995 to 2000.

S = Sandveld: Clanwilliam (6), Graafwater (3), Klipfontein (1), Lambertsbay (1), Piketberg (4), Redelinghuys (10), Van Rhynsdorp (1), Unknown (24)

EC = Eastern Cape: Cradock (1), Hankey (4), Patensie (5), Unknown (1)

LP = Limpopo Province: Alldays (1), Dendron (1), Hoedspruit (1), Modimolle (1), Polokwane (1), Tolwe (2), Vivo (2)

SWC = South-western Cape: Cape Flats (1), Worcester (3)

KZN = KwaZulu-Natal: Boston (1), Ithala Valley (1), Umlaas Road (1)

MP = Mpumalanga: Groblersdal (1), Marble Hall (2)

C = Ceres: Ceres (5)

SC = Southern Cape: George (2)

NW = Northwest: Lichtenburg (1)

WFS = Western Free State: Bultfontein (1), Ventersburg (1)

EFS = Eastern Free State: Fouriesburg (1)

NC = Northern Cape: Barkley-west (1)

G = Gauteng: Pretoria (1)

NEC = North-eastern Cape.

Table 2.1. Relative virulence of selected *Verticillium nigrescens* isolates on potato cultivar BP1.

Isolate no.	Production area	Severity index[a]	Rating
96	South-western ape	2.8 b	Virulent
69	Mpumalanga	3.4 c	Virulent
1	Southern Cape	3.9 cd	Virulent
2	Northern Cape	4.0 d	Virulent
Control		1.5[b] a	

[a]Mean of 10 replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=0.5917).
[b]Natural senescence.

Table 2.2. Relative virulence of selected *Verticillium dahliae* isolates on potato cultivar BP1.

Isolate no.	Production area	Severity index[a]	Rating
13	Ceres	4.6 c	Highly virulent
64	Ceres	4.6 c	Highly virulent
98	Eastern Cape	4.0 bc	Virulent
66	Eastern Cape	4.6 c	Highly virulent
72	Eastern Cape	4.7 cd	Highly virulent
77	Eastern Cape	4.7 cd	Highly virulent
78	Eastern Cape	4.9 cd	Highly virulent
101	Eastern Cape	4.9 cd	Highly virulent
67	KZN	3.5 b	Virulent
68	KZN	4.5 c	Highly virulent
103	Limpopo Province	4.0 bc	Virulent
29	Limpopo Province	4.8 cd	Highly virulent
73	Limpopo Province	5.1 cd	Highly virulent
75	Northwest	5.0 cd	Highly virulent
8	Sandveld	3.3 b	Virulent
16	Sandveld	3.3 b	Virulent
12	Sandveld	3.7 bc	Virulent
97	Sandveld	3.7 bc	Virulent
34	Sandveld	3.8 bc	Virulent
3	Sandveld	4.4 c	Highly virulent
11	Sandveld	4.4 c	Highly virulent
99	Sandveld	4.4 c	Highly virulent
4	Sandveld	4.5 c	Highly virulent
7	Sandveld	4.6 c	Highly virulent
14	Sandveld	4.6 c	Highly virulent
10	Sandveld	4.8 cd	Highly virulent
15	Sandveld	4.8 cd	Highly virulent
51	Sandveld	4.8 cd	Highly virulent
5	Sandveld	4.9 cd	Highly virulent
6	Sandveld	4.9 cd	Highly virulent
39	Sandveld	4.9 cd	Highly virulent
60	Sandveld	4.9 cd	Highly virulent
9	Sandveld	5.1 cd	Highly virulent
38	Sandveld	5.4 d	Highly virulent
76	Southern Cape	4.6 c	Highly virulent
61	South-western Cape	4.6 c	Highly virulent
37	South-western Cape	5.0 cd	Highly virulent
Control		1.5[b] a	

[a]Mean of 10 replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=0.7749).
[b]Natural senescence.

A situation similar to the above occurred in Peru, where chlorosis, defoliation and wilting of potato originally were ascribed to *V. albo-atrum* (Bazan de Segura, 1958), though a subsequent investigation (Martin, 1985) yielded only *V. dahliae* from diseased plants. In Australia, which is climatologically more related to South Africa, both species have been reported from potato, although *V. dahliae* is considered to be the main cause of *Verticillium* wilt (Walker, 1990). In neighbouring Zimbabwe, however, *V. albo-atrum* appears to be the only species associated with the disease (Whiteside, 1966). No information is available on the etiology of *Verticillium* wilt of potato in the rest of Africa.

The other pathogenic species isolated from potato samples, *V. nigrescens*, is recorded here for the first time in South Africa. It is an extremely common soil fungus in Europe and Canada (Isaac, 1953, 1967). However, although having the same optimal growth temperature as *V. dahliae* and a slightly higher maximum (Hoes, 1971; Melouk & Horner, 1974), it has seldom been reported from soils in warmer climates (Domsch *et al.*, 1980). In mints (*Mentha* spp.), attack by *V. nigrescens* has provided the plants with immunity to infection by *V. dahliae* (Melouk & Horner, 1975). Considering the absence of *V. nigrescens* in samples from the Sandveld and Eastern Cape, where a high incidence of *V. dahliae* was evident, a converse relationship may exist in potato. Unlike *V. dahliae*, *V. nigrescens* appears to be stimulated in soil cropped to grasses (Domsch *et al.*, 1980) and crop rotation programmes with grass species aimed at managing *V. dahliae* could therefore selectively benefit *V. nigrescens*.

From the survey it is evident that pathogenic species of *Verticillium* are present in most of the potato-production regions of South Africa, the only possible exceptions being Gauteng and North-eastern Cape. In accordance with literature (Robinson *et al.*, 1957; Isaac & Harrison, 1968; Schnathorst, 1981), the dominant species, *V. dahliae*, was more virulent than the less prevalent *V. nigrescens*. However, almost a third of the isolates could not be identified. As none of them were screened for virulence it is not known if any were pathogenic. Until this has been resolved the etiology of *Verticillium* wilt of potato in South Africa remains inconclusive.

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CHAPTER 3

SCREENING OF NEW SOUTH AFRICAN POTATO CULTIVARS FOR RESISTANCE TO *VERTICILLIUM DAHLIAE*

ABSTRACT

Ten South African potato cultivars, eight of which have recently been released, were evaluated over two seasons in a greenhouse for resistance to *Verticillium dahliae.* The cultivars Aviva, BP1, Bravo, Buffelspoort, Caren, Hoëvelder and Ropedi were classified as susceptible to *Verticillium* wilt, whereas Calibra, Dawn and Devlin were rated as very susceptible. No resistance or tolerance was evident.

INTRODUCTION

Developing genetically stable resistant or tolerant cultivars is the most efficient, economical, and environmentally sound approach to control *Verticillium* wilt in potato (*Solanum tuberosum* L.) (Rowe *et al*, 1987; Nachmias *et al*, 1990; Powelson & Rowe, 1993; Tsor & Nachmias, 1995; Plasencia & Banttari, 1997). Plant resistance to *Verticillium* wilt is defined as inability of the pathogen to penetrate the roots, interruption in host tissue colonisation, inactivation of toxic elements, or suppression of fungal sporulation. A gene conferring resistance to *Verticillium dahliae* Kleb. race 1, referred to as *Ve,* has been identified in tomato (*Lycopersicon esculentum* Mill.) (Uys, 1996), but apparently does not exist in potato (Tsror & Nachmias, 1995). Certain potato varieties, however, show delayed or reduced colonisation after penetration by *V. dahliae* but do not suffer from severe wilt symptoms or a reduction in the marketable yield of tubers, and are therefore considered tolerant (Tsor & Nachmias, 1995; Plasencia & Banttari, 1997).

Traditional screening for resistance to *Verticillium* wilt involves growing potential germplasm in infested soil (Corsini *et al*, 1988; Arbogast *et al*, 1999). Critical evaluation of potato germplasm and clones for resistance to *V. dahliae* under field conditions is time-consuming and expensive. Disease symptoms are not reliable indicators since the characteristic unilateral chlorosis and necrosis typical of *Verticillium* wilt resemble those of natural senescence, and their expression is influenced by environmental variables (Isaac & Harrison,

1968; Nachmias *et al*, 1990; Plasencia & Banttari, 1997). Further complications occur because of the wide range in genotype maturity existing in potato germplasm. While early-maturing resistant clones may appear to be susceptible when they are simply senescing, late-maturing susceptible genotypes might show no symptoms at the time they are evaluated and would be classified as resistant. Allowance therefore has to be made for the effects of maturity in order to obtain more meaningful estimates of disease resistance (Nachmias *et al*, 1990; Plasencia & Banttari, 1997).

Field observations indicated that established cultivars, such as BP1, Up-to-Date and Buffelspoort, which comprise 73 % of all potatoes planted in South Africa, are all susceptible to *Verticillium* wilt. However, various new varieties have been released on the local market since 1988, particularly in 1995 and 1997 (Nortje *et al.*, 2000). The purpose of this study was to evaluate some of these releases for their response to artificial infection with *V. dahliae*.

MATERIALS & METHODS

Eight recently released potato cultivars (Table 3.1) were compared with the established cultivars, BP1 and Buffelspoort, in separate experiments in 2000 and 2001. For each experiment, inoculum of three isolates of *V. dahliae* (nos 38, 60 and 61, Chapter 2) was prepared on V8 juice-supplemented vermiculite as described by Denner (1997). Inoculum of the three isolates was pooled and incorporated at 10 g 1.9 kg^{-1} into a 3:1 (v/v) mixture of tyndallised (105 °C for 30 minutes on three consecutive days) sandy soil (7 % clay) and vermiculite (247 and 392 microsclerotia g^{-1} in 2000 and 2001, respectively). The artificially infested soil was dispensed into 15-cm diameter plastic pots. Ten pots were each planted to a minituber of each of the cultivars in Table 3.1. Planting took place on 4 September in 2000 and on 24 April in 2001. Ten pots without *V. dahliae* inoculum were included as control for each cultivar. Fertiliser (2:3:2 (22) N:P:K) was applied at 1 g per pot at planting. Pots with plants were maintained at 25±2 °C and were irrigated three times a day for 2 minutes by means of an automated micro-irrigation system.

Plants were examined fortnightly from 8 weeks until 14 weeks after planting for the presence of wilting. Stems were divided into three equal sections (± 30 cm each) and a class value assigned to each plant according to a 5-point scale adapted from Robinson *et al.* (1957) and Isaac & Harrison (1968):

1 = no wilting or yellowing

2 = wilting and yellowing in one third of the stem

3 = wilting and yellowing in two thirds of the stem
4 = total wilting and yellowing
5 = whole plant dead

Stem isolations were made from plants, 14 weeks after planting, on PDA supplemented with 100 mg streptomycin sulphate suspended in 10 ml ethanol l^{-1}. Plates were incubated at 25 °C for 3 – 5 days and examined microscopically for the presence of *V. dahliae*.

Classification of accessions into *Verticillium* wilt reaction categories was based on the index of Corsini *et al.* (1988), calculated as follows:

$$\frac{[\{(\text{presence of wilt symptoms 0 or 1}) \times (\text{wilt severity 1-5 scale})\} + (\text{presence of pathogen 0 or 1})]}{\text{median time (weeks after planting) for symptoms to appear}} \times 10$$

Based on differences in index values in infested soil and the corresponding control treatment, the cultivars were classified as follows:

≤ 0.99	=	resistant
1.00 – 2.99	=	susceptible
≥ 3.0	=	very susceptible

A one-way analysis of variance (ANOVA) using the statistical program GENSTAT (2000) was performed to test for differences in disease index between the treatments (cultivars) for each season. Data were acceptably normal with homogeneous treatment variances. Treatment means were separated using Fishers' protected *t*-test least significant difference at 1% level of significance if the F-probability from the ANOVA was significant at 1%.

Results were compared with data from the potato breeding programme at ARC-Roodeplaat regarding resistance of the various cultivars to other diseases such as common scab (*Streptomyces scabies*), early blight (*Alternaria solani* Sorauer) and late blight (*Phytophthora infestans* (Mont.) de Bary), as well as the yield potential of each of the cultivars, adapted from Nortje *et al.* (2000) (Table 3.1).

Table 3.1. *Verticillium* wilt reaction, resistance to other diseases, and yield potential of selected South African potato cultivars.

Cultivar	Maturation period	*Verticillium* wilt reaction[a]		Disease resistance[b]			Yield (t ha^{-1})[b]	
		Spring 2000	Autumn 2001	Common scab[c]	Early blight[d]	Late blight[e]	Spring planting	Autumn planting
Ropedi	Short	1.25 a S	1.73 a S	S	0.25	1.00	60.8	28.2
Buffelspoort	Short	1.48 a S	2.05 a S	VS	1.75	2.50	50.2	29.7
Caren	Medium	2.08 a S	1.70 a S	MT	1.00	1.25	72.3	25.5
Hoëvelder	Long	1.95 a S	1.90 a S	MT	1.00	2.00	75.6	42.8
Aviva	Short	1.75 a S	2.10 a S	T	1.50	1.25	50.1	25.2
BP1	Medium	1.93 a S	2.20 ab S	VS	1.50	2.00	62.8	30.4
Bravo	Long	2.17 ab S	2.18 ab S	MT	1.25	1.50	76.3	35.2
Calibra	Medium	3.20 b VS	2.98 b S	T	2.00	0.75	66.2	29.1
Devlin	Short	2.75 ab VS	4.03 c VS	MT	1.75	1.25	50.7	27.3
Dawn	Medium	3.53 b VS	3.53 bc VS	S	1.75	1.25	50.5	30.6

[a] Mean of 10 replicates; disease indices calculated according to Corsini *et al.* (1988) and with control values subtracted; values in columns followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=1.042 Spring 2000; LSD=0.867 Autumn 2001); S = susceptible, VS = very susceptible.

[b] Adapted from Nortje *et al.* (2000).

[c] MT = moderately tolerant, S = susceptible, T = tolerant, VS = very susceptible.

[d] 0 = no visible leaf infection, 4 = > 75 % of foliage affected.

[e] 0 = no visible stem or leaf infection, 4 = > 75 % of foliage affected.

RESULTS AND DISCUSSION

In the spring 2000 experiment, the cultivars Calibra and Dawn were significantly more susceptible than Aviva, BP1, Buffelspoort, Caren, Hoëvelder and Ropedi (Table 3.1). In the autumn 2001 experiment, Devlin was significantly more susceptible than all other cultivars except Dawn, while the latter and Calibra were significantly more susceptible than Aviva, Buffelspoort, Caren, Hoëvelder and Ropedi. On average, the cultivars Dawn, Devlin and Calibra, could be rated as very susceptible, and the remaining cultivars as susceptible, to *Verticillium* wilt.

The above ratings accurately reflect the inherent susceptibility of the various cultivars to *Verticillium* wilt as they account for natural senescence and are derived from two sets of results. A cultivar that showed delayed or reduced colonisation by *V. dahliae* after penetration but did not exhibit severe wilt symptoms would have been considered tolerant. However, all the cultivars were aggressively colonised by the pathogen and none could therefore be rated in this category. Inoculum density apparently played a minor role in this regard since the 59 % higher microsclerotium density in the 2001 experiment increased the mean disease index by only 10 %.

Selection of the newly released potato cultivars for inclusion in the study depended on their availability at the time of the experiments. Together, the eight new cultivars that were screened represent less than half of the 10% "other" potato genotypes presently planted in South Africa. Considering that none of them proved to be less susceptible than the established cultivars BP1 and Buffelspoort, they cannot be recommended as substitutes if *Verticillium* wilt is the only consideration. All of them nevertheless have acceptable yield potential and other characteristics that could determine their selection. For instance, Calibra, which rated very susceptible to *Verticillium* wilt, is one of only a few cultivars with tolerance to common scab and late blight, though it is susceptible to early blight. Susceptibility of BP1 and Buffelspoort to the latter three diseases will obviously count against them, particularly if *Verticillium* wilt is also present. In such a situation Ropedi seems to be the best option. However, eventual selection would depend on field trials in which the entire complex of agronomic traits such as yield, distribution of tuber size and tuber appearance, as well as resistance to various diseases can be evaluated simultaneously (Corsini & Pavek, 1996).

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CHAPTER 4

CONTROL OF *VERTICILLIUM* WILT OF POTATO BY SOIL INCORPORATION OF BROCCOLI RESIDUES

ABSTRACT

The efficacy of broccoli volatiles *in vitro* on mycelial growth of *Verticillium dahliae*, and the effect of incorporation of fresh and dry broccoli residues on the survival of microsclerotia of *V. dahliae* and infection of potato, were determined in the laboratory and greenhouse. Volatiles emanating from freshly harvested macerated broccoli leaves were inhibitory to mycelial growth of *V. dahliae* on medium. Fresh and dry residues incorporated into soil artificially infested with *V. dahliae,* significantly reduced the viability of microsclerotia of the pathogen and the rate of infection of potato plants. Dry residues were more effective than fresh residues in reducing the viability of sclerotia, but suppression of infection was independent of the state of the residues.

INTRODUCTION

Verticillium wilt of potato (*Solanum tuberosum* L.) caused by *Verticillium dahliae* Kleb. may be either suppressed or controlled by a variety of environmentally-compatible procedures, including resistant cultivars (Davis, 1985; Rowe *et al.*, 1987; Nachmias *et al.*, 1990; Powelson & Rowe, 1993; Tsror & Nachmias, 1995; Davis *et al.*, 1996), long-term crop rotation (Joaquim *et al.*, 1988; Easton *et al.*, 1992; Powelson & Rowe, 1993; Cappaert *et al.*, 1994; Subbarao *et al.*, 1995; Subbarao & Hubbard, 1996; Xiao & Subbarao, 1998; Xiao *et al.*, 1998; Arbogast *et al.,* 1999) and cultural practices that involve incorporation of organic amendments (Easton *et al.*, 1992; Powelson & Rowe, 1993; Subbarao *et al.*, 1995; Davis *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Arbogast *et al.,* 1999; Subbarao *et al.,* 1999; Blok *et al.*, 2000).

An area that is currently being actively researched is biofumigation, i.e. the use of brassicaceous crops to control disease (Subbarao & Hubbard, 1996; Brown & Morra, 1997). Disease control is ascribed to the chemical breakdown of glucosinolates, sulphur-containing compounds responsible for the inherent pungent odour of brassicaceous plants (Mayton *et*

al., 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999; Vaughn, 1999). Glucosinolates are a group of secondary sulphur compounds composed of a thioglucose group, a variable carbon side chain (R-group) and a sulphonated oxide (Mayton *et al.*, 1996; Smolinska *et al.*, 1997; Vaughn, 1999). Glucosinolates are named according to their R-group (Mayton *et al.*, 1996). During the decomposition of brassica residues, glucosinolates, contained in vacuoles, are hydrolysed by the enzyme myrosinase (α-thioglucosidase glucohydrolase), which is present in the cell walls, endoplasmic reticulum, Golgi vesicles and mitochondria, to various biologically active hydrolysis products including isothiocyanates, thiocyanates, nitriles, epinitriles and sulphides. Some of the hydrolytic breakdown products have either fungistatic or fungicidal, antimicrobial and insecticidal properties (Mayton *et al.*, 1996; Subbarao & Hubbard, 1996; Smolinska *et al.*, 1997; Subbarao *et al.*, 1999; Vaughn, 1999). The end-product of the hydrolytic reaction is determined by the R-group of the glucosinolate and the physical and chemical conditions under which hydrolysis takes place. Allyl glucosinolate is generally converted to allyl isothiocyanate (AITC) at a pH of 4.0 or higher. AITC, a volatile compound, is as toxic to fungi as methyl isothiocyanate, the active ingredient in commercial soil fumigants such as dazomet and metham-sodium (Tomlin, 1994; Mayton *et al.*, 1996; Smolinska *et al.*, 1997). AITC breaks the disulphide bond of cystine in proteins and glutathione through oxidative cleavage/scission (Vaughn, 1999). Allyl isothiocyanate production in brassicaceous crops increases with increasing levels of sulphur nutrition in soil (Subbarao & Hubbard, 1996). Approximately 100 different glucosinolates have been identified in plant tissue from at least 11 different plant families, principally the Brassicaceae, Capparidaceae and Resedaceae (Mayton *et al.*, 1996; Vaughn, 1999). As many as 15 different glucosinolates can be produced by a single species, and concentrations of individual glucosinolates vary within different organs of the same plant and within populations of the same species (Vaughn, 1999). The concentration of glucosinolates is highest in actively growing tissues and declines as the plant ages (Matthiessen *et al.*, 2000). Thus, varying types and amounts of glucosinolates within the brassica species determine the level of plant pathogen suppression (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999).

Chapters 2 and 3 of this dissertation have indicated that *V. dahliae* is present in most of the potato-producing areas in South Africa and that resistance to the pathogen apparently does not exist in local potato cultivars. Control of *V. dahliae* in cauliflower (*Brassica oleracea* L. var. *botrytis* L.) has been achieved through soil incorporation of broccoli (*B. oleracea* L. var. *italica* Plenck) residues (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999; Shetty *et al.*,

2000). Thus, developing rotations with broccoli and incorporating broccoli residues into the soil may be a novel way of controlling *Verticillium* wilt of potato (Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Vaughn, 1999). The present study evaluates the potential of six broccoli cultivars to control *V. dahliae* on this crop.

MATERIALS & METHODS

The efficacy of broccoli volatiles on mycelial growth of *V. dahliae* was determined by a modification of the bioassay described by Mayton *et al.* (1996). The effect of incorporating fresh and dry broccoli residues on the survival of microsclerotia of *V. dahliae* and on development of *Verticillium* wilt was ascertained in the laboratory and greenhouse, respectively, according to procedures recommended by Subbarao & Hubbard (1996).

a) Efficacy of broccoli volatiles on mycelial growth of *V. dahliae*:

One half of a 9-cm-diameter split plate, containing potato-dextrose agar (PDA), was inoculated with a 5-mm disc from a 10-day old culture of *V. dahliae* isolate nr. 38 (Chapter 2). Five grams of macerated tissue of freshly harvested broccoli (cvs Dynasty, Green fall, Green king, Kashamari, Liberty or RX1140) was placed in the other half of the split plate. Split plates without broccoli residue served as control. Each treatment was replicated five times. Plates were sealed with cling wrap (crystal clear polyethylene) and aluminium foil, and incubated upright at 25 °C. Colony diameters were determined after 11 days.

A one-way analysis of variance (ANOVA) was conducted on the data. Data were acceptably normal with homogeneous treatment variances. Treatment means were separated using Fishers' protected *t*-test least significant difference (LSD) at 1 % level of significance if the F-probability from the ANOVA was significant at 1 %.

b) Survival of microsclerotia of *V. dahliae* in soil:

Inoculum of three isolates of *V. dahliae* (nos 38, 60 and 61, Chapter 2) was prepared on V8 juice-supplemented vermiculite as described by Denner (1997). Inoculum of the three isolates was pooled and incorporated at 600 g 1.8 kg^{-1} into a 3:1 (v/v) mixture of tyndallised (105 °C for 30 minutes on three consecutive days) sandy soil (7 % clay) and vermiculite.

Twenty-three grams of the *V. dahliae*-infested soil mixture was placed into each of fifty six Consol jars (100 ml volume) and amended with the following: (1) 8% (m/m) fresh residue of each of the above six broccoli cultivars, (2) equivalent mass of dry (45 °C for 6 days) residue of each of the cultivars. An unamended control was included for each condition (fresh or dry residue). Each treatment consisted of four replicates. The soil in each jar was saturated to field capacity. Jars were incubated at 25 °C for 15 days. Viable microsclerotia before and after treatment were enumerated according to the method of Harris *et al.* (1993). The number of microsclerotia per gram of soil was determined as follows: the soil from each bottle was air-dried at room temperature for 7 days, sieved through a 2 mm mesh sieve, and suspended in 100 ml distilled water in an 250 ml Erlenmeyer flask. The suspension was homogenised by vigorous agitation for about 1 hour on a reciprocating shaker. The suspension was washed with tap water through a 90 and 25 μm mesh sieve (20 cm diameter) in series, and the material retained on the 25 μm sieve was returned to the original flask and resuspended in 100 ml 0.1 % water agar. The suspension was agitated thoroughly before withdrawing aliquots of 1 ml soil suspension and transferring each to three plates of modified soil-extract agar (MSEA). Plates were incubated at 25 °C for 4 weeks in the dark, and the soil was removed by washing with tap water. Using a dissecting microscope, plates were observed for colonies of *V. dahliae* at 25 x magnification. Identity of the colonies were verified on cornmeal agar (CMA) plates. The number of viable microsclerotia per gram of soil was determined as follows: mean number of colonies on the three plates / (mass of soil sample / volume of 0.1 % water agar used). The survival of microsclerotia after treatment was expressed as a ratio of the number of microsclerotia present before treatment.

A two-way analysis of variance was conducted on the ratio of survival of microsclerotia to test for differences between the treatments (control + six cultivars), condition (fresh and dry residue) and the treatment–by–combination interaction effect. Data were acceptably normal with homogeneous treatment variances. Treatment means were separated using Fishers' protected *t*-test least significant difference (LSD) at 1 % level of significance if the F-probability from the ANOVA was significant at 1 %.

c) *Verticillium* wilt of potato:

Inoculum of three isolates of *V. dahliae* (nos 38, 60 and 61, Chapter 2) was prepared on V8-juice supplemented vermiculite as described by Denner (1997). Inoculum of the three isolates was pooled and incorporated at 10 g 1.9 kg^{-1} into a 3:1 (v/v) mixture of tyndallised (105 °C for 30 minutes on three consecutive days) sandy soil (7% clay) and vermiculite (192 microsclerotia g^{-1} soil). The artificially infested soil was dispensed into 15-cm diameter plastic pots.

Ten pots with artificially infested soil were each planted to one of the above broccoli cultivars. Fertiliser (1 g 2:3:2 (22) N: P: K) was applied at planting to each pot. Pots were arranged in a completely randomised block design on a bench in a greenhouse at 25 ± 2 °C. Plants were irrigated three times a day for 2 minutes by means of an automated micro-irrigation system. Five pots without broccoli, served as control. Broccoli heads were harvested after 90 days and the residues (outer leaves) were removed, macerated, and a portion dried in an oven at 45 °C for 6 days. Half of the pots previously planted to broccoli were amended with 8 % (m/m) fresh residue of the same cultivar as planted before, and the other half with the equivalent mass of dry residue. A potato minituber (cv. BP1) was planted to each pot 30 days after incorporation of broccoli residues. Fertiliser (1 g 2:3:2 (22) N: P: K per pot) was again applied at planting and plants were irrigated as before. Stem isolations were made from all the plants on PDA as described in Chapter 2, 12 weeks after inoculation. Plates were incubated at 25 °C for 3 to 5 days and examined microscopically for the presence of *V. dahliae*.

A two-way analysis of variance was conducted on the presence of *V. dahliae* in stems to test for differences between the treatments (control + 6 cultivars), condition (fresh and dry residue) and the treatment–by–combination interaction effect. Data were acceptably normal with homogeneous treatment variances. Treatment means were separated using Fishers' protected *t*-test least significant difference (LSD) at 1 % level of significance if the F-probability from the ANOVA was significant at 1 %.

RESULTS

a) Efficacy of broccoli volatiles on mycelial growth of *V. dahliae*:

Volatiles from all broccoli cultivars significantly, and to the same extent, suppressed mycelial growth of *V. dahliae* (Fig. 4.1; Table 4.1).

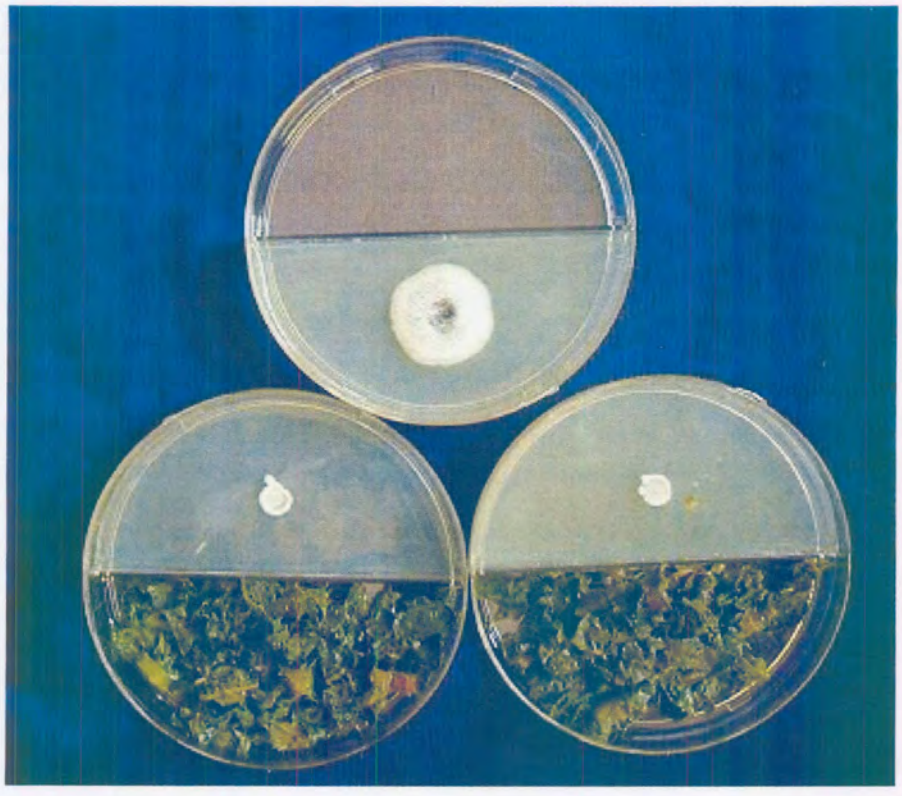

Fig. 4.1. Growth of *Verticillium dahliae* on one half of a PDA split plate containing 5 g of freshly macerated residues of the broccoli cultivar Kashamari (bottom) or no residues (top), after 11 days.

Table 4.1. Effect of broccoli volatiles on growth of *Verticillium dahliae* on potato dextrose agar.

Broccoli cultivar	Colony diameter after 11 days (mm) [a]
Control	16.2 a
Green fall	2.9 b
Green king	2.9 b
Dynasty	2.4 b
Liberty	2.3 b
Kashamari	2.0 b
RX1140	2.0 b

[a]) Mean of five replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=1.289).

b) Survival of microsclerotia in soil:

Fresh and dry residues of all broccoli cultivars significantly, and to the same extent, reduced microsclerotial viability (Table 4.2, Fig. 4.2). The reduction with dry residues was significantly ($P < 0.01$) greater than with fresh residues, viz. 94 % versus 57 % (Fig. 4.3).

c) *Verticillium* wilt of potatoes:

Incorporation of fresh and dry residues of all broccoli cultivars significantly reduced infection of potato stems by *V. dahliae* (Table 4.3). However, there was no significant difference in the reduction of infection between dry and fresh residues. The cultivars Green fall and RX1140 were the most effective in reducing infection (Fig. 4.4).

DISCUSSION

This study has shown that volatiles released from freshly harvested macerated broccoli leaves are inhibitory to growth of *V. dahliae* isolated from potato, and that fresh and dry broccoli residues incorporated into soil reduce the viability of microsclerotia of the pathogen and infection of potato plants. Dry residues were more effective than fresh ones in devitalising microsclerotia, though the two types of residues reduced infection to the same extent. This is in conflict with Subbarao & Hubbard (1996) and Subbarao *et al.* (1999) who found fresh broccoli residues to be considerably more effective than dry residues for control of *Verticillium* wilt in cauliflower. The reason for the difference in response may be ascribed to the difference in drying time of residue (2 days versus 6 days in the present study). Nevertheless, reduction in disease is more important than reduction in inoculum in disease control, and both fresh and dry residues can therefore be recommended for managing *Verticillium* wilt of potato.

In accordance with Subbarao *et al.* (1999), preliminary results of the present study (data not presented) indicated that reduction in *V. dahliae* microsclerotia do not occur during the growth of a broccoli crop. Matthiessen *et al.* (2000), on the other hand, claimed that roots might release isothiocyanate during growth as well as during decomposition. The mechanisms by which crucifer residues act on plant pathogens are assumed to be mostly chemical. Because most glucosinolate breakdown products are volatile their retention in the soil environment is very short. Microsclerotia of *V. dahliae*, however, survive in soil for prolonged periods of time. Thus, a transient exposure to the volatile gases may be insufficient to affect the viability of a significant number of *V. dahliae* microsclerotia. As the microsclerotia are located both in the soil and in association with organic debris, they are not

Table 4.2. Ratio of survival of microsclerotia of *Verticillium dahliae* in artificially infested soil amended with fresh or dry residues of six broccoli cultivars, 15 days after incorporation of the residues.

Broccoli cultivar	Fresh residue[a]	Dry residue[a]	Mean of treatments[b]
Control	0.830	0.613	0.721 a
Liberty	0.540	0.073	0.306 b
Green king	0.557	0.015	0.286 b
Dynasty	0.430	0.080	0.255 b
RX1140	0.340	0.155	0.248 b
Kashamari	0.350	0.025	0.188 b
Green fall	0.340	0.013	0.176 b
Mean of condition	0.484 a	0.139 b	

[a])Mean of four replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=0.0722).

[b])Mean of eight replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference ($P \leq 0.01$; LSD=0.1350).

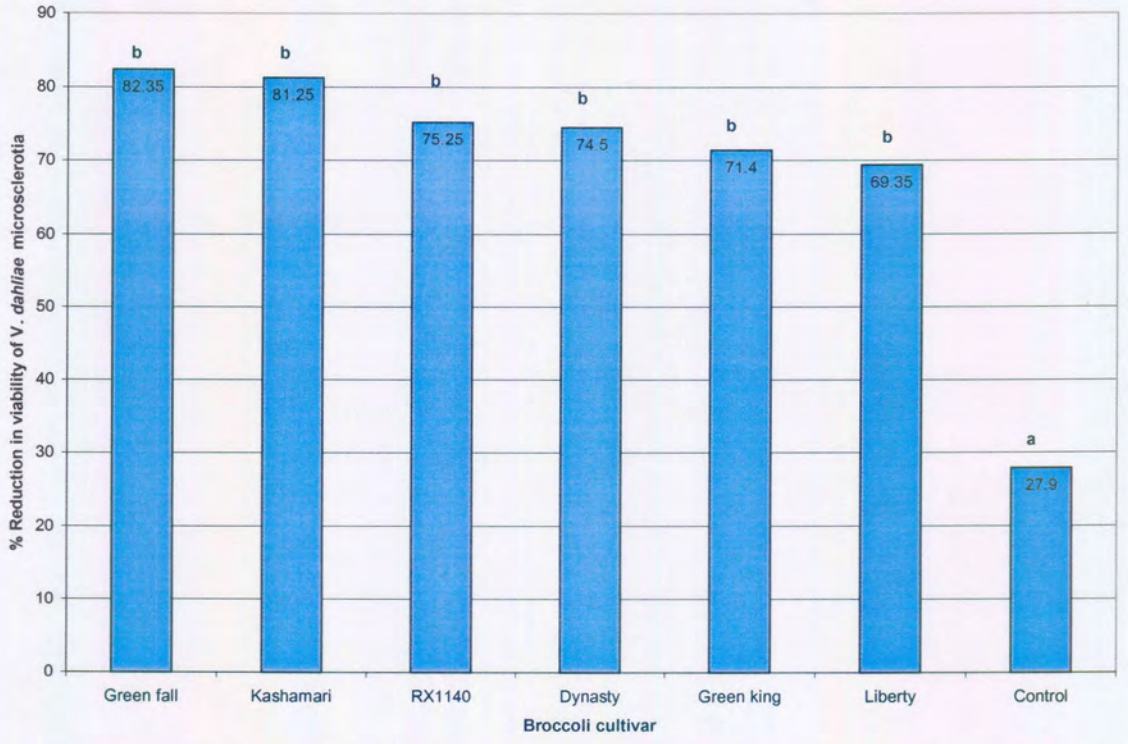

Fig. 4.2. Percentage reduction in viability of microsclerotia of *Verticillium dahliae* in artificially infested soil amended with fresh or dry residues of six broccoli cultivars, 15 days after incorporation of the residues. Bars with the same letter do not differ significantly according to Fishers' protected t-test least significant difference (P≤0.01; LSD=0.1350).

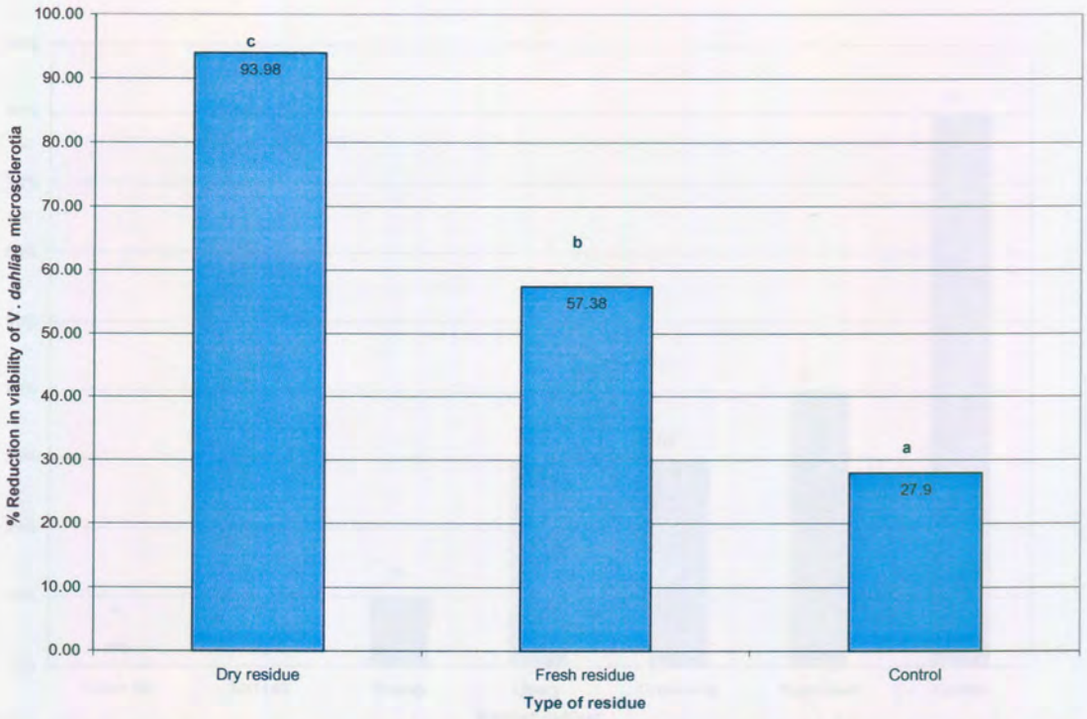

Fig. 4.3. Percentage reduction in viability of microsclerotia of *Verticillium dahliae* in artificially infested soil amended with fresh or dry broccoli residues, 15 days after incorporation of the residues. Bars with the same letter do not differ significantly according to Fishers' protected t-test least significant difference (P≤0.01; LSD=0.0722).

Table 4.3 The presence of *Verticillium dahliae* in stems of potato plants in soil artificially infested with the pathogen, and planted to and amended with fresh or dry broccoli residues, 30 days after incorporation of the residues.

Broccoli cultivar	Fresh residue	Dry residue	Mean of treatments[a]
Control	1.8	1.8	1.80 a
Kashamari	1.60	1.20	1.40 b
Green king	1.00	1.60	1.30 bc
Liberty	1.40	1.20	1.30 bc
Dynasty	1.00	1.20	1.10 bc
Green fall	1.00	1.00	1.00 c
RX1140	1.00	1.00	1.00 c

[a]Mean of ten replicates; values followed by the same letter do not differ significantly according to Fishers' protected *t*-test least significant difference (P≤0.01; LSD=0.33).

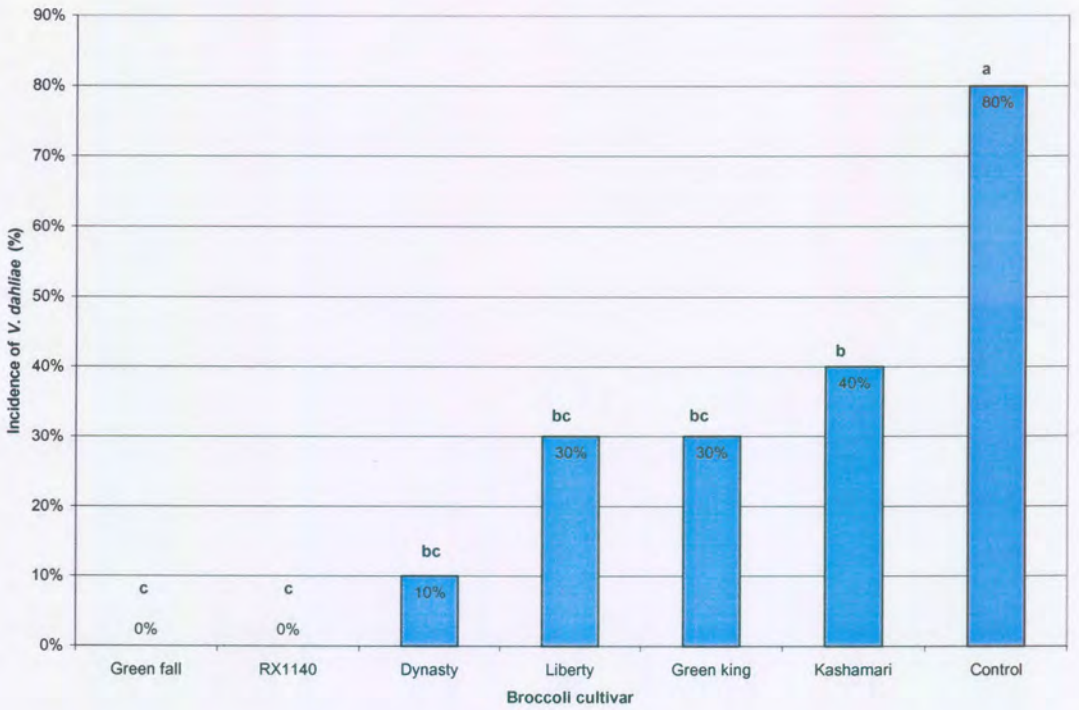

Fig. 4.4 Incidence of *Verticillium dahliae* in stems of potato plants in soil artificially infested with the pathogen, and planted to and amended with fresh or dry broccoli residues, 30 days after incorporation of the residues. Bars with the same letter do not differ significantly according to Fishers' protected t-test least significant difference (P≤0.01; LSD=0.33).

uniformly exposed to the volatile gases. It is quite possible that other biological mechanisms in broccoli-amended soil might affect pathogen propagule survival (Subbarao & Hubbard, 1996). Incorporation of rotation crop residues (green manures) provides an alternative approach to disease control, as it could increase nutrient availability, reduce groundwater contamination, and stimulate beneficial microflora in soil, including antagonists that inhibit *V. dahliae* (Easton *et al.*, 1992; Davis *et al.*, 1996). Increased microbial activity following broccoli amendment and the resulting competition for colonisation of root cortical surface may limit infection loci for *V. dahliae*.

Broccoli is a versatile crop with culinary and medicinal value, and also has deleterious effects on various plant pathogens (Subbarao & Hubbard, 1996). Considering the efficacy of biofumigation with other brassicaceous crops on potato pathogens such as *Colletotrichum*

coccodes (Wallr.) S. Hughes, *Fusarium sambucinum* Fuckel, *Helminthosporium solani* Dur. & Mont., *Phytophthora cryptogea* Pethybr. & Laff., *P. erythroseptica* Pethybr., *Rhizoctonia solani* J.G. Kühn (AGs 3 and 8) and *Streptomyces scabies* (Vaughn, 1999, Gouws & Mienie, 2000; Harding & Wicks, 2000), broccoli has the potential to control these organisms as well. On the other hand, although broccoli is known to resist attack by *V. dahliae* (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999; Shetty *et al.*, 2000), it is host to a number of important potato pathogens, e.g. *Erwinia carotovora* and *Sclerotinia sclerotiorum* (Lib.) de Bary (Howard *et al.*, 1994; Crous *et al.*, 2000), and can therefore sustain their numbers in soil when rotated with potato.

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CHAPTER 5
GENERAL DISCUSSION

Subsequent to the frst report of *Verticillium* wilt of potato (*Solanum tuberosum* L.) in South Africa by Doidge (1950), no new cases were confirmed until 1989. The increased number of recordings since 1989 showed *Verticillium* wilt to be of major concern to the potato industry (Millard, 1999). The survey conducted in this study showed *Verticillium* wilt of potato in South Africa to be caused by *Verticillium dahliae* Kleb. mainly. Managing a persistent pathogen like *V. dahliae* with the ability to produce microsclerotia is not readily achievable. Due to the expected withdrawal of methyl bromide that will leave fewer fumigants for effective management of *Verticillium* wilt, and the recent emphasis on sustainable agriculture, the world-wide trend is to focus on an integrated control strategy to suppress or control *Verticillium* wilt of potato (Davis & Sorensen, 1986; Davis *et al.*, 1996; Subbarao & Hubbard, 1996; Xiao *et al.*, 1998; Subbarao *et al.*, 1999; Blok *et al.*, 2000). The various control procedures should be implemented before and after planting of the crop. A feasible integrated control strategy for the control of *Verticillium* wilt of potato in South Africa is presented in Fig. 5.1.

A) Pre-planting

1) Seed selection

As a vascular pathogen, *V. dahliae* can colonise tubers through stolons and remain present as dormant mycelium in the vascular tissues (Nachmias *et al.*, 1982; Rowe, 1985; Nagtzaam *et al.*, 1997). Therefore, especially in a seed-certification scheme, it is important to be able to detect the pathogen by means of a rapid, sensitive detection method (Nachmias *et al.*, 1982; Plasencia *et al.*, 1996). Furthermore, as infected seed stock serves as a primary source of inoculum to uninfested soil, it is imperative never to use seed tubers coming from fields with a history of *Verticillium* wilt (Rowe, 1985; Rowe *et al.*, 1987).

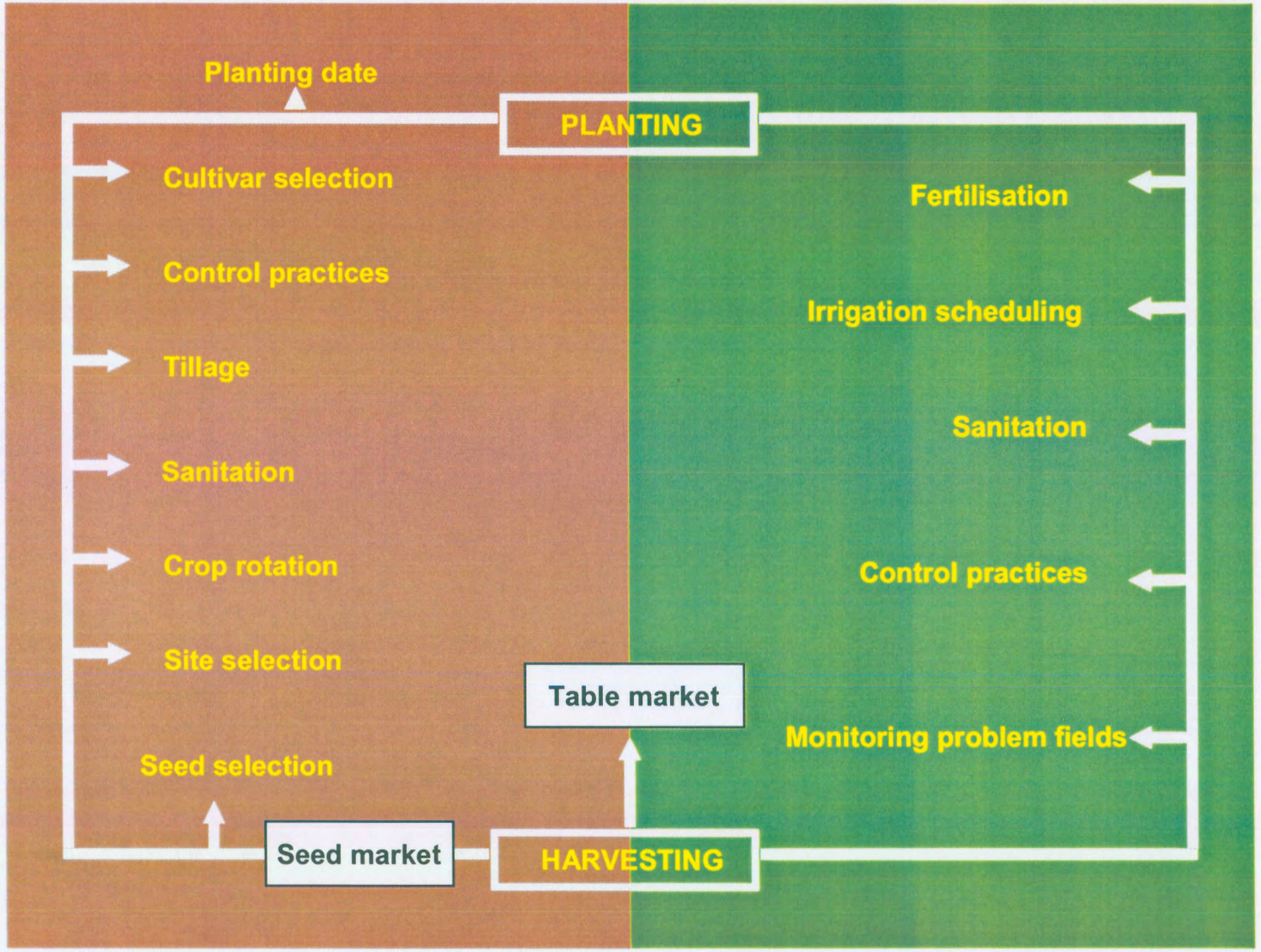

Fig. 5.1. Proposed integrated control strategy for the control of *Verticillium* wilt of potato in South Africa.

2) Site selection

The key to managing *Verticillium* wilt is to reduce the number of microsclerotia in soil to levels too low to cause disease in susceptible cultivars. It is therefore important to determine the number of viable *V. dahliae* microsclerotia in soil to do a disease risk assessment of possible planting sites as well as to evaluate the performance of soil disinfestation procedures (Rowe *et al.*, 1987; Termorshuizen, 1998).

3) Crop rotation

Barley (*Hordeum vulgare*. L.), bluegrass (*Poa* spp.), carrot (*Daucus carota* L.), maize (*Zea mays* L.), mung bean (*Vigna radiata* (L.) R. Wilcz), wheat (*Triticum aestivum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), and sugar beet (*Beta vulgaris* L.) are potential rotation crops considered nonhosts of *V. dahliae* (Easton *et al.*, 1992). Short crop rotations rarely are effective in eradicating *V. dahliae*, because of the slow attrition rate of microsclerotia in soil, inoculum density levels well above economic threshold at the onset of rotation, and wide host range of the pathogen. Furthermore, germinating microsclerotia may colonise roots of nonsusceptible weeds or crops such as oats (*Avena sativa* L.), maize, wheat, barley and grain sorghum at low levels, thereby maintaining inoculum densities sufficient to infect susceptible hosts (Harrison & Isaac, 1969; Joaquim *et al.*, 1988; Easton *et al.*, 1992; Mol *et al.*, 1996; Xiao & Subbarao, 1998; Xiao *et al.*, 1998). Broccoli has the potential as a rotation and green manure crop to reduce microsclerotia of *V. dahliae* in soil and *Verticillium* wilt of potato as part of an integrated control strategy by virtue of its biofumigation capacity (Subbarao & Hubbard, 1996; Subbarao *et al.*, 1999).

4) Sanitation

Sanitation is important in preventing the introduction of the pathogen into wilt-free fields and in reducing losses from wilt in infested fields (El-Zik, 1985). Thus, proper disposal of infected plant parts and debris that may harbour the pathogen, such as the removal or burning of potato vines, can reduce the severity of the disease and spread of inoculum to "new" potato fields and fields that recently have been fumigated (Davis, 1985; El-Zik, 1985; Rowe *et al.*, 1987; Powelson & Rowe, 1993). Furthermore, implements and equipment used to prepare the soil for planting or other operations, as well as shoes, should always be properly cleaned and disinfested to avoid spread of inoculum to soil free of *V. dahliae* (El-Zik, 1985).

5) Tillage

Propagules of *V. dahliae* are most prevalent in the plant bed and top 30 cm of soil. Deep ploughing, particularly where the soil is completely inverted, can be effective in reducing disease losses (El-Zik, 1985). Soil type is a key factor in the success of deep ploughing, as it will not be effective in sandy soils because of the difficulty to completely invert such soils (Millard & Denner, 2001).

6) Control practices

Verticillium wilt may be controlled by a variety of fumigation treatments that have biocidal activity, such as methyl bromide, chloropicrin and metham-sodium, but costs limits the widespread application of these products (Davis, 1985; El-Zik, 1985). Chemical fumigants also may have undesirable effects on beneficial organisms, such as mycorrhizal fungi, in the rhizosphere (El-Zik, 1985).

Soil solarisation, a hydrothermic process that occurs in moist soil covered with transparent polyethylene sheeting during periods of high solar radiation, can reduce the number of viable microsclerotia in soil greatly. However, a suitable climate is essential for solarisation to be successful (Tjamos & Jimenez-Diaz, 1998). Furthermore, the treatment is not practical on a large scale.

Seed treatment for the control of tuberborne diseases like black dot (*Colletotrichum coccodes* (Wallr.) S. Hughes) and black scurf (*Rhizoctonia solani* J.G. Kühn) reduces plant stress and therefore susceptibility to *Verticillium* wilt (Millard & Denner, 2001). Proper nematode control is important because *V. dahliae* and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven interact synergistically. Together they cause severe symptoms and yield reductions, even at densities that separately would have little or no effect (Rowe *et al.*, 1987: Powelson & Rowe, 1993). In South Africa, *P. penetrans* is not the dominantlesion nematode on potato, and the interaction of *V. dahliae* with species of *Pratylenchus* more common in the country, e.g. *P. brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven (Van den Berg, 1971), needs to be investigated.

An effective weed control programme helps to reduce the incidence of *Verticillium* wilt, because several weed species, such as black nightshade (*Solanum nigrum* L.), common purslane (*Portulaca oleracea* L.), pigweed (*Amaranthus deflexus* L.),

shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) and species in the genera *Chenopodium, Lamium* and *Medicago,* growing in and around fields, may serve as alternative hosts for *V. dahliae*. Development of microsclerotia in senescent tissues of infected weeds could increase the inoculum levels in soil and thus negate disease control obtained by rotation with a nonsusceptible crop (El-Zik, 1985; Vargas-Machuca *et al.*, 1987).

7) Cultivar selection

Although the development of genetically-stable resistant or tolerant cultivars is considered to be the most efficient, economical, and environmentally sound approach to control *Verticillium* wilt in potato worldwide, all the local cultivars tested thus far proved to be susceptible to *Verticillium* wilt.

8) Planting date

Potatoes grow optimally within a temperature range of 18-20 °C. The optimum range for growth of *V. dahliae,* however, is 21-27 °C. Reflecting these temperature optima, disease severity in potatoes infected with *V. dahliae* tends to increase as the mean air temperature rises from 20-28 °C (Powelson & Rowe, 1993)

B) Post-planting

1) Fertilisation

Balanced nutrition of major elements (nitrogen, phosphorus and potassium) and minor elements is essential for minimising plant stress and susceptibility to *Verticillium* wilt. Fertilisation should therefore be limited to levels for optimal yield, with pre-plant soil analysis as foundation (El-Zik, 1985; Davis & Everson, 1986).

2) Irrigation scheduling

Water management early in the season is recommended, because high soil water content during tuber initiation increases infection, whereas low soil water content after infection, enhances symptom expression (Cappaert *et al.*, 1992, 1994).

3) **Sanitation**

As mentioned earlier, sanitation is important in preventing the introduction of the pathogen into wilt-free fields and in reducing losses from wilt in infested fields (El-Zik, 1985).

4) **Control practices**

Efficient control of foliar diseases such as late blight (*Phytophthora infestans* (Mont.) de Bary) and early blight (*Alternaria solani* Sorauer) will reduce plant stress and therefore susceptibility to *Verticillium* wilt (Millard & Denner, 2001).

5) **Monitoring potato fields**

It is essential to monitor diseases appearing on potatoes during the growing season in each field, especially *Verticillium* wilt, because of the survival of microsclerotia of *V. dahliae* in soil for long periods in the absence of a host.

An effective disease management system of *Verticillium* wilt of potato implies an orderly and planned strategy involving the use of several approaches, implemented at various times during the potato crop cycle, and integrated into the overall crop production system. Therefore, efforts to control *Verticillium* wilt of potato that involve several options and tactics will be more enduring and effective than those relying on a single option.

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Verticillium wilt of potato in South Africa

by

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RESUMÉ

Since the first report of *Verticillium* wilt of potato in 1950, the disease has been considered to be of minor importance in South Africa. Between 1995 and 2000, however, *Verticillium* spp. were isolated from 146 samples of symptomatic potato plant material received from 13 of the 14 potato production areas in the country. Of 93 *Verticillium* isolates that were obtained, 60 % were identified as *V. dahliae* and 8 % *V. nigrescens*. *V. dahliae* was present in nine of the regions and *V. nigrescens* in seven. Unidentified *Verticillium* species were isolated from six of the regions. Both *V. dahliae* and *V. nigrescens* were pathogenic to potato *in vivo*, with *V. dahliae* the more virulent of the two species.

Ten South African potato cultivars, eight of which have recently been released, were evaluated over two seasons in a greenhouse for resistance to *V. dahliae*. The cultivars Aviva, BP1, Bravo, Buffelspoort, Caren, Hoëvelder and Ropedi were classified as susceptible to *Verticillium* wilt, whereas Calibra, Dawn and Devlin were rated as very susceptible. No resistance or tolerance was evident.

The efficacy of broccoli volatiles on *in vitro* mycelial growth of *Verticillium dahliae*, and the effect of incorporation of fresh and dry broccoli residues on the survival of microsclerotia of *V. dahliae* and infection of potato, were determined in the laboratory and greenhouse. Volatiles emanating from freshly harvested macerated broccoli leaves were inhibitory to mycelial growth of *V. dahliae* on medium. Fresh and dry residues incorporated into soil artificially infested with *V. dahliae*, significantly reduced the viability of microsclerotia of the pathogen and the rate of infection of potato plants. Dry residues were more effective than

fresh residues in reducing the viability of sclerotia, but suppression of infection was independent of the state of the residues.

Verticillium-verwelk van aartappels in Suid-Afrika

deur

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SAMEVATTING

Sedert die eerste aanmelding van *Verticillium*-verwelk van aartappels in 1950, is die siekte nie as van belang in Suid-Afrika beskou nie. Gedurende die tydperk 1995 tot 2000 is *Verticillium* spp. egter vanuit 146 simptomatiese plantmateriaalmonsters, afkomstig vanuit 13 van die 14 aartappel-produserende streke in die land, geïsoleer. Uit 93 *Verticillium* isolate wat bekom is, is 60 % as *V. dahliae* en 8 % as *V. nigrescens* geïdentifiseer. *V. dahliae* en *V. nigrescens* was onderskeidelik in nege en sewe van die streke teenwoordig. Ongeïdentifiseerde *Verticillium* species was in nege van die streke teenwoordig. *In vivo* toetse het getoon dat beide *V. dahliae en V. nigrescens* patogenies is op aartappels, met *V. dahliae* die virulentste van die twee spesies.

Tien Suid-Afrikaanse aartappelkultivars, waarvan agt onlangs vrygestel is, is oor twee seisoene in 'n glashuis geëvalueer vir bestandheid teen *V. dahliae*. Die kultivars Aviva, BP1, Bravo, Buffelspoort, Caren, Hoëvelder en Ropedi is as vatbaar vir *Verticillium*-verwelk geklassifiseer, terwyl Calibra, Dawn en Devlin geblyk het baie vatbaar te wees. Geen bestandheid of verdraagsaamheid is waargeneem nie.

Die effektiwiteit van vlugtige stowwe op *in vitro* miseliumgroei van *V. dahliae*, sowel as die effek van inkorporering van vars en droë broccoli reste op die oorlewing van mikrosklerotiums van *V. dahliae* en infeksie van aartappels, is in die laboratorium en glashuis bepaal. Vlugtige stowwe afkomstig vanaf vars-geoeste, gekerfde broccoli blare het 'n inhiberende effek op miseliumgroei van V. *dahliae* op medium gehad. Vars en droë broccoli reste, geïnkorporeer in grond wat kunsmatig met *V. dahliae* besmet is, het die

lewensvatbaarheid van *V. dahliae* mikrosklerotiums sowel as die mate van infeksie van aartappelplante, betekenisvol verlaag. Droë reste het die lewensvatbaarheid van mikrosklerotiums meer effektief as vars reste verlaag, maar onderdrukking van infeksie was onafhanklik van die tipe residu.