VERTICILLIUM WILT OF POTATO IN SOUTH AFRICA

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SUMMARY

Subsequent to the first 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). In a survey conducted between 1995 and 2000, Verticillium spp. was 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 (Appendix A).

As a vascular pathogen, Verticillium spp. can colonize tubers through stolons and remain present as dormant mycelium in the vascular tissues (Nachmias et al, 1982; Nagtzaam et al, 1997; Rowe, 1985). Therefore, especially in a seed certification-scheme, it is important to be able to detect this systemic plant pathogen by means of rapid, sensitive detection methods (Nachmias et al, 1982; Plasencia et al, 1996). Conventional methods for detection and identification are laborious and time-consuming (Plasencia et al, 1996). An alternative approach would be to use serological procedures such as enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich (DAS)-ELISA is especially useful for detecting antigens in complex mixtures, such as soil or plant extracts, because the bound antibody specifically captures the antigen(s) of interest, while irrelevant material is removed in the initial wash step (Sundaram et al, 1991). Two (DAS)-ELISA- kits supplied by BIOREBA AG in Switzerland and AGDIA were evaluated for the sensitivity and specificity of each kit. The BIOREBA kit seems to be more sensitive than the AGDIA kit for the detection of V. dahliae and V. nigrescens while the AGDIA kit seems to be the more consistent kit. Due to the fact that both ELISA-kits were sensitive for the presence of F. solani and/or F. oxysporum, it is unsuitable for the use as a standard method to detect mycelium in infected tuber material in a seed certification scheme. Fusarium wilt also causes vascular browning similar to Verticillium wilt. It is therefore important to test other molecular techniques for the rapid and accurate detection of V. dahliae in infected tuber material (Appendix B).

Considering the prolonged survival of microsclerotia, a key to managing Verticillium wilt is to reduce the number of microsclerotia in soils to levels too low to cause disease in susceptible crops. Field trials showed that the threshold value for disease might be as low as 0.5 colony-forming units g⁻¹ soil (Appendix C).

This accentuates the importance of sanitation to prevent the introduction of the pathogen into wilt-free fields and in reducing losses from wilt in infested fields. 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. 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. There are several chemical products that can be successfully used for sanitation of implements, equipment, cold rooms, floors, shoes, etc (Appendix D).

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, was 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 (Appendix E).

The principal source of inoculum of *V. dahliae* is microsclerotia (resting structures), which can survive in soil for up to 13 years in the absence of a host. *Verticillium dahliae* can survive on roots and stems of many weed species such as nightshade, small flowered quickweed, sowthistle, suring, tall khakiweed, wandering jew, wild bindweed, white-flowered mexican poppy, and yellow nutsedge. It is therefore important to control weeds effectively to prevent serious outbreaks that may well result from large increases in the populations of propagules already present in the soil due to intensive cultivation of highly susceptible host plants such as potatoes (Appendix F).

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 worldwide. During 2000 and 2001, 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 (Appendix G). During 2003 to 2005, 13 local potato cultivars were evaluated for resistance to *Verticillium* wilt over 3 seasons. The cultivars Evan, Eryn, Hertha, Mnandi, Mondial and Ronn were classified as moderate susceptible, while the cultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell and Up-to-date, were classified as susceptible to *Verticillium* wilt (Appendix H). It is important to keep in mind that these results are based on greenhouse trials. Final selection would depend on field trials in which the entire complex of agronomic traits such as yield, distribution of tuber size and tuber appearance, can be evaluated simultaneously.

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 Figure 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) (Appendix B). 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).
Figure 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. Field trials showed that the threshold value for disease might be as low as 0.5 colony-forming units g⁻¹ soil (Appendix C). 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.), 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). 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, was 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 (Appendix E).

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). There are several chemical products that can be successfully used for sanitation of implements, equipment, cold rooms, floors, shoes, etc (Appendix D).

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 dominant lesion 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, common purslane, pigweed, shepherd’s purse, small flowered quickweed, sowthistle, suring, tall khakiweed, wandering jew, wild bindweed, white-flowered mexican poppy, yellow nutsedge and species in the genera *Chenopodium*, *Lamium* and *Medicago*, growing in and around fields, may serve as alternative hosts for *V. dahliae* (Appendix F). 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). It is therefore important to control weeds effectively to prevent serious outbreaks that may well result from large increases in the populations of propagules already present in the soil due to intensive cultivation of highly susceptible host plants such as potatoes.

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. 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 (Appendix G). During 2003 to 2005, 13 local potato cultivars were evaluated for resistance to *Verticillium* wilt over 3 seasons. The cultivars Evan, Eryn, Hertha, Mnandi, Mondial and Ronn were classified as moderate susceptible, while the cultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell and Up-to-date, were classified as susceptible to *Verticillium* wilt (Appendix H).
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 involves several options and tactics will be more enduring and effective than those relying on a single option.
REFERENCES


APPENDIX A

OCCURRENCE AND DISTRIBUTION OF VERTICILLIUM SPECIES ON POTATO IN SOUTH AFRICA

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 10⁶ 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):

\[((\text{presence of wilt symptoms, 0 or 1}) \times (\text{wilt severity, 1-5})) + (\text{re-isolation of pathogen, 0 or 1})\]

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. 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 1). Of the 37 V. dahliae isolates screened, eight were virulent and 29 highly virulent (Table 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. 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 1. Relative virulence of selected *Verticillium nigrescens* isolates on potato cultivar BP1.

<table>
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<th>Isolate no.</th>
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<td>69</td>
<td>Mpumalanga</td>
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<td>Virulent</td>
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<td>1</td>
<td>Southern Cape</td>
<td>3.9 cd</td>
<td>Virulent</td>
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<td>2</td>
<td>Northern Cape</td>
<td>4.0 d</td>
<td>Virulent</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1.5$^b$ a</td>
<td></td>
</tr>
</tbody>
</table>

$^a$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. Relative virulence of selected *Verticillium dahliae* isolates on potato cultivar BP1.

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

*Mean of 10 replicates; values followed by the same letter do not differ significantly according to Fishers’ protected t-test least significant difference (P≤0.01; LSD=0.7749).

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.

**REFERENCES**

INTRODUCTION

Verticillium wilt of potatoes is a vascular disease occurring in both irrigated and non-irrigated production areas of the world, resulting in yield reduction (Arbogast et al., 1999; Davis & Sorenson, 1986; Plasencia et al., 1996; Tsor and Nachmias, 1995). Verticillium spp., the causal organism of Verticillium wilt, is extremely common and can be found in many cultivated soils. Contamination of uninfested fields with Verticillium spp. can occur by wind or mechanical movement of soil particles containing viable propagules, but a primary method is introduction on infected seed stock. As a vascular pathogen, Verticillium spp. can colonize tubers through stolons and remain present as dormant mycelium in the vascular tissues (Nachmias et al., 1982; Nagtzaam et al., 1997; Rowe, 1985). Therefore, especially in a seed certification-scheme, it is important to be able to detect this systemic plant pathogen by means of rapid, sensitive detection methods (Nachmias et al., 1982; Plasencia et al., 1996). Conventional methods for detection and identification are laborious and time-consuming (Plasencia et al., 1996). An alternative approach would be to use serological procedures such as enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich (DAS)-ELISA is especially useful for detecting antigens in complex mixtures, such as soil or plant extracts, because the bound antibody specifically captures the antigen(s) of interest, while irrelevant material is removed in the initial wash step (Sundaram et al., 1991).

An ELISA-kit supplied by BIOREBA AG in Switzerland was tested in co-operation with Coen Bezuidenhout test laboratories for the efficiency of detecting conidia of V. dahlieae in a conidial suspension, as well as detecting V. dahlieae in tubers and stems of artificial infected glasshouse plants. The ELISA-kit showed potential for the detection of V. dahlieae in plant tissue of potato plants. Further studies were done to determine the sensitivity of the technique as well as the specificity of this kit to detect mycelium in infected material.

MATERIALS & METHODS

1. Two (DAS)-ELISA- kits supplied by BIOREBA AG in Switzerland and AGDIA were evaluated for the sensitivity and specificity of each kit. The efficiency of detecting conidia of V. dahlieae, V. nigrescens, and Fusarium solani isolates in different conidial suspensions varying between 1 x 10^5 and 1 x 10^6 conidia/ml were determined in duplicate according to manufacturers instructions. The absorbance values were determined after 30 and 60 minutes of incubation, with a Multiskan Plus P using filter 405. Values ≥ 0.1 were considered to be positive.

2. The experiment was repeated but this time V. dahlieae, V. nigrescens, Fusarium solani, F. oxysporum, and Colletotrichum coccodes isolates with conidial suspensions varying between 1 x 10^1, 1 x 10^2, 1 x 10^3, 1 x 10^4 and 1 x 10^5 conidia/ml were used. The absorbance values were determined after 60 minutes of incubation, with a Multiskan Plus P using filter 405. Values ≥ 0.1 were considered to be positive.

3. The experiment was repeated but this time 3 isolates each of V. dahlieae, V. nigrescens, and Fusarium solani with conidial suspension of 1 x 10^5 conidia/ml were used. A sub-treatment where plant juice was added or not added to each well was included. The absorbance values were determined after 60 minutes of incubation, with a Multiskan Plus P using filter 405. Values ≥ 0.1 were considered to be positive.
4. The experiment was repeated but this time 2 isolates each of *V. dahliae*, and *V. nigrescens* with conidial suspensions of $1 \times 10^4$ and $1 \times 10^7$ conidia/ml were used. A subtreatment where plant juice was added or not added to each well was included. The absorbance values were determined after 60 minutes of incubation, with a Multiskan Plus P using filter 405. Values $\geq 0.1$ were considered to be positive.

**RESULTS**

**Experiment 1:**
The absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for $1 \times 10^5$ and $1 \times 10^6$ conidia/ml conidial suspensions of *V. dahliae*, *V. nigrescens*, and *Fusarium solani*. are shown in Table 1. 

Sensitivity:
Both the Agdia and Bioreba kits detected the $1 \times 10^5$ and $1 \times 10^6$ conidia/ml suspensions of *V. dahliae* after 30 as well as 60 minutes.

Specificity:
Both the Agdia and Bioreba kits are not specific. 

*V. nigrescens*: Both the Agdia and Bioreba kits detected the $1 \times 10^5$ conidia/ml suspension after 60 minutes. Only the Bioreba kit detected the $1 \times 10^6$ conidia/ml suspension after 30 as well as 60 minutes.

*F. solani*: Both the Agdia and Bioreba kits detected the $1 \times 10^5$ and $1 \times 10^6$ conidia/ml suspensions after 60 minutes.

Control: Both the Agdia and Bioreba kits showed high positive absorbance values for the positive control after 30 as well as 60 minutes. Both kits showed high positive absorbance values for the negative control after 60 minutes, while the Bioreba kit showed some positive values after 30 minutes as well.

Background coloring:
Bioreba: The high column mean blank value as well as the high positive values for the negative control after 60 minutes indicates a high background reaction. Furthermore, absorbance values ranging between 0.007 and 0.528 were obtained in the 6 blank cells after 30 minutes, and –0.006 and 0.973 after 60 minutes.

Agdia: The high positive values for the negative control after 60 minutes, may be indicative of a background reaction.

**Experiment 2:**
The absorbance values obtained after 60 minutes with the Bioreba and Agdia kits for $1 \times 10^1$ to $1 \times 10^5$ conidia/ml conidial suspensions of *V. dahliae*, *V. nigrescens*, *Fusarium solani*, *F. oxysporum*, and *C. coccodes*. are shown in Table 2.

Sensitivity:
The Agdia kit detected *V. dahliae* only in the $1 \times 10^4$ and $1 \times 10^5$ conidia/ml conidial suspension treatment, while the Bioreba kit detected the presence of *V. dahliae* in all the treatments.

Specificity:
Both the Agdia and Bioreba kits are not-specific.

*V. nigrescens*: The Agdia kit did not detected the pathogen but the Bioreba kit detected the $1 \times 10^3$ conidia/ml conidial suspension.

*F. solani*: The Agdia kit did not detected the pathogen but the Bioreba kit detected the pathogen in all except the $1 \times 10^1$ conidia/ml conidial suspension.

*F. oxysporum*: The Agdia kit detected the pathogen in the $1 \times 10^5$ conidia/ml conidial suspension while the Bioreba kit detected the pathogen in all except the $1 \times 10^1$ conidia/ml conidial suspension.

*C. coccodes*: No one of the kits detected the pathogen.

Background coloring: Both the Agdia and Bioreba kits showed positive absorbance values in the column mean blank indicating a high background reaction.
Experiment 3:
The absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for $1 \times 10^5$ conidia/ml conidial suspensions of 3 isolates each of *V. dahliae*, *V. nigrescens*, and *Fusarium solani* are shown in Table 3. Specificity:
Both of the kits were non-specific.
*V. dahliae*: The Agdia kit detected isolate 38 after 30 minutes when plant juice was added and after 60 minutes when plant juice was not added, isolate 60 after 60 minutes when plant juice was added, and isolate 73 was not detected at all. The Bioreba kit didn't detect any of the 3 isolates.
*V. nigrescens*: The Agdia kit detected all 3 isolates after 30 minutes when plant juice was added and isolate 69 and 96 after 60 minutes when plant juice was not added. The Bioreba kit only detected isolate 96 after 30 as well as 60 minutes when no plant juice was added.
*F. solani*: The Agdia kit only detected isolate 1 after 30 minutes when plant juice was added and after 60 minutes when plant juice was not added. The Bioreba kit only detected isolate 2 after 30 as well as 60 minutes when no plant juice was added.
Control: Both kits detected the positive control after 30 and 60 minutes. The Agdia kit showed only one positive absorbance value in the negative control after 60 minutes, but in comparison with the high values of the positive control it may be overspill from the positive control. No one of the kits detected the juice control.

Experiment 4:
The absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for $1 \times 10^4$ and $1 \times 10^7$ conidia/ml conidial suspensions of 2 isolates each of *V. dahliae*, and *V. nigrescens* are shown in Table 4. Sensitivity:
The Agdia kit detected isolate 73 of *V. dahliae* at $1 \times 10^7$ after 60 minutes when no plant juice was added, and isolate 38 at $1 \times 10^7$ after 60 minutes although plant juice was added or not. The Bioreba kit detected both isolates at both concentrations after 30 as well as 60 minutes although plant juice was added or not.
Specificity:
The Agdia kit detected isolate 57 of *V. nigrescens* at $1 \times 10^7$ after 30 as well as 60 minutes when no plant juice was added. The Bioreba kit detected both isolates at both concentrations after 60 minutes although plant juice was added or not.
Control: Both kits detected the positive control after 30 as well as 60 minutes. The Bioreba kit showed positive absorbance values for the negative and the juice control after 30 minutes and high positive absorbance values after 60 minutes.
Background coloring: The high positive absorbance values of the negative and juice control as well as the high absorbance values of 0.868 and 0.898 of the 2 blank cells of the Bioreba kit after 60 minutes, indicates a high background reaction.

DISCUSSION
The BIOREBA kit seems to be more sensitive than the AGDIA kit for the detection of *V. dahliae* because it detected the pathogen in conidial suspensions from as low as $1 \times 10^7$ conidia/ml in contrast with the AGDIA kit that detected it only from the $1 \times 10^5$ conidia/ml conidial suspensions. Because the Agdia kit detected *V. dahliae* in all 4 experiments, in contrast with the Bioreba kit that didn't detected it during experiment 3, although the positive control was detected, it seems to be the more consistent kit. Verticillium wilt of potatoes in South Africa is caused by *V. dahliae* as well as *V. nigrescens*, although *V. dahliae* are the major causal organism. Both of the kits were able to detect *V. nigrescens*. Just like for the detection of *V. dahliae*, the Bioreba kit was more sensitive than the Agdia kit for the detection of *V. nigrescens*. Due to the fact that both ELISA-kits were sensitive for the presence of *F. solani* and/or *F. oxysporum*, it is unsuitable for the use as a standard method to detect mycelium in infected tuber material in a seed certification scheme. *Fusarium* wilt also causes vascular browning similar to Verticillium wilt. It is therefore important to test other molecular techniques for the rapid and accurate detection of *V. dahliae* in infected tuber material. Although both kits
showed a high background reaction, the Bioreba kit seems to be more likely to show a high background reaction. It is recommended that an ELISA-kit supplied by BIOREBA AG in Switzerland should be tested in co-operation with Coen Bezuidenhout test laboratories for the efficiency of detecting *V. dahliae* in post control tubers.

REFERENCES

Table 1. Absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for 1 x $10^5$ and 1 x $10^6$ conidia/ml conidial suspensions of *V. dahliae*, *V. nigrescens*, and *Fusarium solani*.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Concentration</th>
<th>Replicate</th>
<th>A30</th>
<th>A60</th>
<th>B30</th>
<th>B60</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x $10^5$</td>
<td>1</td>
<td>0.106</td>
<td>2.847</td>
<td>0.157</td>
<td>2.804</td>
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<tr>
<td><em>V. dahliae</em></td>
<td>1 x $10^5$</td>
<td>2</td>
<td>0.091</td>
<td>2.781</td>
<td>0.129</td>
<td>2.605</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x $10^6$</td>
<td>1</td>
<td>0.127</td>
<td>2.978</td>
<td>0.204</td>
<td>2.897</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x $10^6$</td>
<td>2</td>
<td>0.112</td>
<td>2.659</td>
<td>0.164</td>
<td>3.158</td>
</tr>
<tr>
<td><em>V. nigrescens</em></td>
<td>1 x $10^5$</td>
<td>1</td>
<td>0.056</td>
<td>0.646</td>
<td>0.072</td>
<td>1.333</td>
</tr>
<tr>
<td><em>V. nigrescens</em></td>
<td>1 x $10^5$</td>
<td>2</td>
<td>0.046</td>
<td>0.531</td>
<td>0.063</td>
<td>0.998</td>
</tr>
<tr>
<td><em>V. nigrescens</em></td>
<td>1 x $10^6$</td>
<td>1</td>
<td>0.068</td>
<td>0.063</td>
<td>0.127</td>
<td>0.117</td>
</tr>
<tr>
<td><em>V. nigrescens</em></td>
<td>1 x $10^6$</td>
<td>2</td>
<td>0.076</td>
<td>0.095</td>
<td>0.132</td>
<td>0.244</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>1 x $10^5$</td>
<td>1</td>
<td>-0.008</td>
<td>1.857</td>
<td>-0.014</td>
<td>3.616</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>1 x $10^5$</td>
<td>2</td>
<td>-0.026</td>
<td>2.723</td>
<td>-0.042</td>
<td>3.107</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>1 x $10^6$</td>
<td>1</td>
<td>-0.011</td>
<td>2.268</td>
<td>-0.015</td>
<td>2.981</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>1 x $10^6$</td>
<td>2</td>
<td>-0.015</td>
<td>2.809</td>
<td>-0.029</td>
<td>2.614</td>
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<tr>
<td>neg. control</td>
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<td>0.06</td>
<td>0.844</td>
<td>0.116</td>
<td>1.46</td>
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<tr>
<td>neg. control</td>
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<td>0.026</td>
<td>0.815</td>
<td>0.063</td>
<td>1.528</td>
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<td>1.287</td>
<td>2.486</td>
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<tr>
<td>pos. control</td>
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<td>1.813</td>
<td>2.854</td>
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<td>column mean blank</td>
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<td>0.174</td>
<td>0.249</td>
<td>0.279</td>
<td>0.409</td>
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</table>
Table 2. Absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for 1 x 10^1 to 1 x 10^5 conidia/ml conidial suspensions of *V. dahliae*, *V. nigrescens*, *Fusarium solani*, *F. oxysporum* and *C. coccodes*.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Concentration</th>
<th>Replicate</th>
<th>A60</th>
<th>B60</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x 10^5</td>
<td>1</td>
<td>0.265</td>
<td>2.892</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x 10^5</td>
<td>2</td>
<td>0.358</td>
<td>2.891</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x 10^5</td>
<td>1</td>
<td>0.015</td>
<td>2.978</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1 x 10^3</td>
<td>1</td>
<td>0.138</td>
<td>2.662</td>
</tr>
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<td><em>V. dahliae</em></td>
<td>1 x 10^4</td>
<td>1</td>
<td>0.221</td>
<td>2.618</td>
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<tr>
<td><em>V. dahliae</em></td>
<td>1 x 10^3</td>
<td>2</td>
<td>0.072</td>
<td>2.486</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
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Table 3. Absorbance values obtained after 30 and 60 minutes with the Bioreba and Agdia kits for 1 x 10^5 conidia/ml conidial suspensions of 3 isolates each of *V. dahliae*, *V. nigrescens*, and *Fusarium solani*.

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Verticillium wilt of potatoes is a vascular disease occurring in both irrigated and non-irrigated production areas of the world, resulting in yield reduction (Arbogast et al., 1999; Davis & Sorenson, 1986; Plasencia et al., 1996; Tsor and Nachmias, 1995). Verticillium spp., the causal organism of Verticillium wilt, is extremely common and can be found in many cultivated soils. Contamination of uninfested fields with Verticillium spp. can occur by wind or mechanical movement of soil particles containing viable propagules, but a primary method is introduction on infected seed stock. As a vascular pathogen, Verticillium spp. can colonise tubers through stolons and remain present as dormant mycelium in the vascular tissues. In spite of the obvious potential for introduction on seed stocks, V. dahliae may not have to be introduced into new lands, but in many cases, may be there naturally on roots of native vegetation. Because of a wide host range, the fungus can survive at low levels on roots of many crop and weed species. In these cases, serious outbreaks may well result from large increases in the populations of propagules already present in the soil due to intensive cultivation of highly susceptible host plants such as potatoes. V. dahliae can survive in soil for more than 10 years in a dormant state as microsclerotia free or embedded in organic debris (Nagtzaam et al., 1997; Rowe, 1985).

Because of the prolonged survival of microsclerotia in soil, a key to managing Verticillium wilt of potatoes is to reduce the number of microsclerotia in soil to levels too low to cause disease (Davis et al., 1996; Subbarao et al., 1999; Subbarao & Hubbard, 1996; Xiao et al., 1998). Therefore, the relationship between inoculum density in soil at planting and wilt development is essential for the development of a disease risk assessment based on preplant soil assays, as well as for the determination of initiation, nature and duration of control practices (Xiao & Subbarao, 1998).

The objectives for this study were to determine the relationship between inoculum density of Verticillium dahliae in soil and (i) onset, (ii) incidence, and (iii) severity of Verticillium wilt of potatoes, as well as (iv) the effect on potato yield.

**MATERIALS & METHODS**

a) **Greenhouse**

Minitubers (UTD) were planted in a greenhouse in a sterile mixture (tindalization at 105°C for 3 alternative days) of sandy soil (7% clay)(Sandland at Roodeplaat) and vermiculite (3:1), and inoculated with soil infested with 1 and 8 microsclerotia/g soil. Inoculation took place six weeks after planting. An uninoculated control was also included. Isolations were made from stems of all plants 8 weeks after inoculation, on Potato Dextrose Agar (PDA) in order to determine the percentage plants infected with V. dahliae.

b) **Field trial**

**Inoculum production**

Individual microsclerotia were produced by incubating sterile vermiculite inoculated with V. dahliae isolates nr. 38 and 60, at room temperature for 2 weeks, and then air-dried for 2 weeks. The number
of microsclerotia per gram of vermiculite was estimated by grinding 1 g subsamples in 10 ml of sterile water. One ml of the suspension was transferred to 9 ml 0.1% wateragar. The process was repeated to establish a dilution series from $1 \times 10^{-1}$ to $1 \times 10^6$. Thereafter, 100 μl aliquots of each dilution were transferred to 3 PDA plates and incubated for 7 days at 25°C in the light. The number of colony forming units per gram of vermiculite were determined as follows: cfu / gram vermiculite = number of colonies on the plate $\times 10^{1+}$ (positive value of number of dilution). An average of 14.4 colonies were counted on the $1 \times 10^{-5}$ dilution plates. Thus, the amount of cfu/g vermiculite is $1.44 \times 10^5$.

**Inoculation of soil**

Micro-plots (9 m x 2 m; Hutton soil) of a field at ARC-Roodeplaat were artificially inoculated with different quantities of inoculated vermiculite to develop different inoculum densities (0, 1, 10, 25, 50, and 100 microsclerotia / g soil). A randomised block design consisting out of 5 replicates was used.

**Plant material**

Double rows of mini-tubers (cv. UTD) were planted 30 cm apart in each micro-plot. The following fertilizers were added: 1300 kg /ha$^1$ 2:3:2 (22) during planting and 90 kg /ha$^1$ KAN 4 weeks after plant. Chemicals for early- and late blight control were also applied when necessary.

**Determination of inoculum density**

Soil was collected 3 days after plant, 6 weeks after plant, and at harvest, 30-45 cm deep with a standard 2.5 cm soil auger and bulked from each corner and the center of a 10 x 10 m quadrat in each treatment. Soil samples were tested according to the method described by Harris et al (1993): The soil samples were air-dried at room temperature for 4 weeks. Four 15g subsamples / sample were sieved through a 2 mm sieve. Each subsample was suspended in 100 ml distilled water in an Erlenmeyer flask. The suspension was broken down by vigorous agitation for about 1 hr on a reciprocating shaker at 270 rpm. The suspension was washed through 90- and 25μm mesh sieves (20 cm diameter) with tap water, and the material on the 25μm sieve was recovered into the original flask, and resuspended in 100 ml 0.1% wateragar. The suspension was shaken thoroughly before withdrawing aliquots of 1ml soil suspension were transferred to 10 plates of modified soil extract agar (MSEA). Plates were incubated at 25°C for 2 weeks in the dark, and the soil was removed by washing with tap water. Plates were further incubated again 2 weeks. Using a dissecting microscope, plates were observed for colonies of V. dahliae at x 25 magnification. Colonies were verified on cornmeal agar (CMA) plates. The number of microsclerotia per gram of soil were determined as follows: Microsclerotia / gram soil = number of positive colonies on plates / [(15 g soil / 100 ml 0.1% water agar ) x 1 ml of solution transferred to MSEA plates].

**Visual evaluation of trail**

The plants were examined fortnightly for symptom expression. Since the characteristic symptoms of unilateral chlorosis and necrosis were morphologically indistinguishable from natural senescence, a symptom and senility index of Isaac and Harrison (1968) was used so that the development of disease and senescence symptoms could be compared graphically. The stems was divided into four equal regions and class values assigned to each on a modified 5 point scale of Robinson et al, 1957 and Isaac and Harrison (1968):

1 = no wilting or yellowing
2 = wilting and yellowing in base of stem
3 = wilting and yellowing in first half of stem
4 = wilting and yellowing in first three-quarters of stem
5 = total wilting and yellowing

The symptom and senility index was calculated as a percentage for each group of plants in a single treatment. The number of stems showing a particular value were multiplied by that value, the products
thus obtained for all the stems were added together and the total multiplied by 100. This figure was then divided by 5 (maximum value of symptoms) times the total number of stems for the treatment. (% symptom and senility index = total of values x 100 / total for treatment x 5). To obtain a single value of relative amount of damage caused, an average index was calculated from the figures of the three readings obtained immediately before onset of rapid senescence in control.

**Vascular colonization**
Every 5 plant in a row were collected. 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. Stem material was divided vertically, and 4 pieces of vascular tissue from 25 stem pieces were transferred to Potato Dextrose Agar plates and incubated for 3-5 days at 25°C. The same procedure was followed for tuber material, except that a thin layer of the stem-end of 50 tubers was removed, and vascular tissue from the remaining part of the tuber was used. Thereafter plates were microscopically examined for the presence of *Verticillium* spp. The number of positive colonies were expressed as a percentage.

**RESULTS**

a) **Greenhouse**
The proportion of stems colonised by *V. dahliae* increased with increasing soil inoculum level of *V. dahliae*. An inoculum level of 1 microsclerotia/g of soil caused 45% wilt incidence, while 8 microsclerotia/g of soil, caused 63% wilt incidence.

b) **Field trial**
No typical yellowing and wilting visual symptoms were found. The percentage vascular colonization of stems and tubers are presented in Table 1.

**DISCUSSION**

It is essential to understand the relationship between inoculum density in soil at planting and wilt development to develop a disease risk assessment based on preplant soil assays. Inoculum density can also be an important factor in determining when control practices should be initiated, their nature, and their duration. The results showed that (i) one microsclerotia per gram soil is sufficient for disease development, (ii) lack of typical visual symptoms of *Verticillium* wilt is not an indication of absence of the disease, and (iii) uninfested soil can easily be infested by mechanical movement of soil particles containing viable propagules (wind, water, shoes, implements, etc.).

**REFERENCES**


### Table 1. Percentage infection of potato mini-tubers (cv. UTD) planted in artificially inoculated soil at different inoculum density levels.

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<th>Percentage infected tubers</th>
<th>Percentage tuber colonization</th>
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<td>4.6</td>
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INTRODUCTION
Sanitation is important in preventing the introduction of the pathogen into wilt-free fields and in reducing losses from wilt in infested fields. 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*.

MATERIAL AND METHODS
Seven chemical products were evaluated *in vitro* against *V. dahliae* at the following dosages: 0, 1, 2, 4, 6 and 8%. Ten ml of a 1 x 10^6 conidia/ml conidial suspension prepared from a 10 day-old *V. dahliae* culture, were added to 90 ml of each of the different dosages of each chemical product. After 1, 2, 5, 10, 30 and 60 minutes exposing periods, 0.1 ml of each dilution was transferred to triplicate Potato Dextrose Agar plates. Plates were incubated at 25°C. Colonies formed were counted after 7 days.

RESULTS
The results of the trial are presented in Table 1.

Table 1. Minimum dosage and exposing period necessary for total control of *V. dahliae* with seven different chemical products

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DISCUSSION
There are several chemical products that can be successfully used for sanitation of implements, equipment, cold rooms, floors, shoes, etc. We do not recommend the use of Organoflo as sanitation product for the control of *V. dahliae*. 
APPENDIX E

CONTROL OF VERTICILLIUM WILT OF POTATO BY SOIL INCORPORATION OF BROCCOLI 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; 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; 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).
Verticillium 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. 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) 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 and 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 Erlenmeyer flask. The suspension was homogenised by vigorous agitation for about 1 hour on a reciprocating shaker. The suspension was washed through a 90 and 25 μm mesh sieve (20 cm diameter) with tap water, and the material
retained on the 25 \( \mu \text{m} \) 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 \textit{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 + 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 %.

c) \textit{Verticillium} wilt of potato

Inoculum of three isolates of \textit{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 of 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 of 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 \textit{V. dahliae}.

A two-way analysis of variance was conducted on the presence of \textit{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 1; Table 1).

b) **Survival of microsclerotia in soil**:
Fresh and dry residues of all broccoli cultivars significantly, and to the same degree reduced microsclerotial viability (Table 2; Fig 2). The reduction with dry residues was significantly (p < 0.01) greater than with fresh residues, viz. 94% versus 57% (Fig 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 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).

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 (two days versus six days during this 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 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, because they 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 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.

REFERENCES


Fig 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 1. Effect of broccoli volatiles on growth of *Verticillium dahliae* on potato dextrose agar.

<table>
<thead>
<tr>
<th>Broccoli cultivars</th>
<th>Colony diameter after 11 days (mm) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.2 a</td>
</tr>
<tr>
<td>Green fall</td>
<td>2.9 b</td>
</tr>
<tr>
<td>Green king</td>
<td>2.9 b</td>
</tr>
<tr>
<td>Dynasty</td>
<td>2.4 b</td>
</tr>
<tr>
<td>Liberty</td>
<td>2.3 b</td>
</tr>
<tr>
<td>Kashamari</td>
<td>2.0 b</td>
</tr>
<tr>
<td>RX1140</td>
<td>2.0 b</td>
</tr>
</tbody>
</table>

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 ≤ 0.01; LSD = 1.289).
Table 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.

<table>
<thead>
<tr>
<th>Broccoli cultivar</th>
<th>Fresh residue</th>
<th>Dry residue</th>
<th>Mean of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.830</td>
<td>0.613</td>
<td>0.721 a</td>
</tr>
<tr>
<td>Liberty</td>
<td>0.540</td>
<td>0.073</td>
<td>0.306 b</td>
</tr>
<tr>
<td>Green king</td>
<td>0.557</td>
<td>0.015</td>
<td>0.286 b</td>
</tr>
<tr>
<td>Dynasty</td>
<td>0.430</td>
<td>0.080</td>
<td>0.255 b</td>
</tr>
<tr>
<td>RX1140</td>
<td>0.340</td>
<td>0.155</td>
<td>0.248 b</td>
</tr>
<tr>
<td>Kashamari</td>
<td>0.350</td>
<td>0.025</td>
<td>0.188 b</td>
</tr>
<tr>
<td>Green fall</td>
<td>0.340</td>
<td>0.013</td>
<td>0.176 b</td>
</tr>
<tr>
<td>Mean of condition</td>
<td>0.484 a</td>
<td>0.139 b</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)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 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 (\(P \leq 0.01\); LSD=0.1350).
Fig 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 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.

<table>
<thead>
<tr>
<th>Broccoli cultivar</th>
<th>Fresh residue</th>
<th>Dry residue</th>
<th>Mean of treatments&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.8</td>
<td>1.8</td>
<td>1.80 a</td>
</tr>
<tr>
<td>Kashamari</td>
<td>1.60</td>
<td>1.20</td>
<td>1.40 b</td>
</tr>
<tr>
<td>Green king</td>
<td>1.00</td>
<td>1.60</td>
<td>1.30 bc</td>
</tr>
<tr>
<td>Liberty</td>
<td>1.40</td>
<td>1.20</td>
<td>1.30 bc</td>
</tr>
<tr>
<td>Dynasty</td>
<td>1.00</td>
<td>1.20</td>
<td>1.10 bc</td>
</tr>
<tr>
<td>Green fall</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 c</td>
</tr>
<tr>
<td>RX1140</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 c</td>
</tr>
</tbody>
</table>

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. 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).
APPENDIX F

ALTERNATIVE HOSTS OF V. DAHLIAE

INTRODUCTION

Verticillium dahliae commonly survive in a dormant state in cultivated soils (Rowe, 1985; Rowe et al., 1987;) as small (30-50 μm), multicelled, melanized hyphae structures called microsclerotia (Menzies & Griebel, 1967; Rowe, 1985; Rowe et al., 1987; Soesanto & Termorshuizen, 2001). Microsclerotia can survive for more than 10 years (Rowe, 1985; Rowe et al., 1987; Easton, 1992; Tjamos & Fravel, 1995; Subbarao & Hubbard, 1996; Xiao & Subbarao, 1998; Xiao et al., 1998; Subbarao et al., 1999; Soesanto & Termorshuizen, 2001) free or embedded in organic debris in soil (Rowe, 1985; Rowe et al., 1987; Tjamos & Fravel, 1995; Nagtzaam et al., 1997; Soesanto & Termorshuizen, 2001) under adverse environmental conditions (Tjamos & Fravel, 1995) without the presence of host plants (Menzies & Griebel, 1967; Schnathorst, 1981, Cappaert et al., 1994; Tjamos & Fravel, 1995). Contamination of uninfested fields can occur by wind or mechanical movement of soil particles containing viable propagules, but a primary method is introduction on infected seed stock. In spite of the obvious potential for introduction on seed stocks, V. dahliae may not have to be introduced into new lands, but in many cases, may be there naturally on roots of native vegetation (Rowe, 1985; Rowe et al., 1987). Due to a wide host range, the fungus can survive at low levels on roots of many crop and weed species (Green, 1980; Rowe, 1985; Rowe et al., 1987; Joaquim et al., 1988; Easton, 1992; Powelson & Rowe, 1993). In these cases, serious outbreaks may well result from large increases in the populations of propagules already present in the soil due to intensive cultivation of highly susceptible host plants such as potatoes (Rowe, 1985; Rowe et al., 1987).

MATERIALS & METHODS

Weeds from a potato field (Roodeplaat) were collected and transferred to 10 pots each in a greenhouse. The soil was artificially inoculated with 1 x 10^7 conidia / ml conidial suspension. Isolations were made from 5 of the plants, 12 weeks after inoculation, on Potato Dextrose Agar (PDA). Plates were incubated for 3-5 days at 25°C. Thereafter plates were microscopically examined for the presence of V. dahliae. The other half of the plants was air-dried for a further 4 weeks. Isolations were made from the dried weed material (roots) on Potato Dextrose Agar (PDA). Plates were incubated for 3-5 days at 25°C. After incubation, plates were microscopically examined for the presence of V. dahliae.
RESULTS
The presence of *V. dahliae* in root, stem and dried material of each of the weeds tested, are presented in Table 12.

Table 12. Percentage plants with *V. dahliae* present in root, stem and dried material of 13 weed species from a potato field.

<table>
<thead>
<tr>
<th>Family</th>
<th>Botanical name</th>
<th>Common name</th>
<th>Algemene naam</th>
<th>Yellowing and wilting symptoms</th>
<th>Fresh root material</th>
<th>Fresh stem material</th>
<th>Dry material</th>
<th>Previously reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apiaceae</td>
<td><em>Hydrocotyle americana</em></td>
<td>navelwort, water pennywort</td>
<td>perdekioutjes</td>
<td>yes</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Argemone ochroleuca</em></td>
<td>white-flowered mexican poppy</td>
<td>mexikaanse papawer</td>
<td>no</td>
<td>0.00%</td>
<td>0.00%</td>
<td>60.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Conyza canadensis</em></td>
<td>horseweed fleabane</td>
<td>kanadese skraaithans</td>
<td>yes</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Galinsoga parviflora</em></td>
<td>small-flowered quickweed, galiant soldier</td>
<td>knopkruid</td>
<td>no</td>
<td>0.00%</td>
<td>0.00%</td>
<td>20.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Sonchus oleraceus</em></td>
<td>sowthistle, milkthistle</td>
<td>sydissel, tuindissel</td>
<td>yes</td>
<td>0.00%</td>
<td>40.00%</td>
<td>20.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Tagetes minuta</em></td>
<td>tall khaki weed, mexican marigold</td>
<td>kakiebos, langkakiebos</td>
<td>no</td>
<td>0.00%</td>
<td>20.00%</td>
<td>20.00%</td>
<td>no</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Taraxum officinalis</em></td>
<td>common dandelion</td>
<td>perdeblom</td>
<td>yes</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
</tr>
<tr>
<td>Commelinaceae</td>
<td><em>Commelina benghalensis</em></td>
<td>benghal-wandering jew, commelina</td>
<td>wandelende jood</td>
<td>yes</td>
<td>20.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
</tr>
<tr>
<td>Convolvulaceae</td>
<td><em>Convolvulus farinosus</em></td>
<td>Wild bindweed</td>
<td>klimop</td>
<td>no</td>
<td>0.00%</td>
<td>0.00%</td>
<td>20.00%</td>
<td>no</td>
</tr>
<tr>
<td>Cyperaceae</td>
<td><em>Cyperus esculentis</em></td>
<td>yellow nutedge, yellow watergrass</td>
<td>geeluiintjie</td>
<td>yes</td>
<td>0.00%</td>
<td>0.00%</td>
<td>20.00%</td>
<td>yes</td>
</tr>
<tr>
<td>Oxalidaceae</td>
<td><em>Oxalis spp</em></td>
<td>suring</td>
<td>no</td>
<td>20.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum nigrum</em></td>
<td>black-nightshade</td>
<td>nastergal, galbessie</td>
<td>yes</td>
<td>0.00%</td>
<td>60.00%</td>
<td>0.00%</td>
<td>yes</td>
</tr>
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<td>Verbenaceae</td>
<td><em>Verbena bonariensis</em></td>
<td>wild verben, purple top</td>
<td>blouwaterbossie</td>
<td>yes</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>no</td>
</tr>
</tbody>
</table>

DISCUSSION

*Verticillium dahliae* can survive on roots and stems of many weed species such as nightshade, small flowered quickweed, sowthistle, suring, tall khakiweed, wandering jew, wild bindweed, white-flowered mexican poppy, and yellow nutsedge. It is therefore important to control weeds effectively to prevent serious outbreaks that may well result from large increases in the populations of propagules already present in the soil due to intensive cultivation of highly susceptible host plants such as potatoes.
APPENDIX G

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 (Tsor & 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; Arbobast 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:

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

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

\[ \leq 0.99 \quad = \quad \text{resistant} \]
\[ 1.00 - 2.99 \quad = \quad \text{susceptible} \]
\[ \geq 3.0 \quad = \quad \text{very susceptible} \]

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).

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).

REFERENCES


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

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Maturation period</th>
<th><em>Verticillium</em> wilt reaction&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Disease resistance&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Yield (t ha&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spring 2000 Autumn 2001</td>
<td>Common scab&lt;sup&gt;c&lt;/sup&gt; Early blight&lt;sup&gt;d&lt;/sup&gt; Late blight&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Spring planting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autumn planting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ropedi</td>
<td>Short</td>
<td>1.25 a S 1.73 a S</td>
<td>S</td>
<td>0.25</td>
</tr>
<tr>
<td>Buffelspoort</td>
<td>Short</td>
<td>1.48 a S 2.05 a S</td>
<td>S</td>
<td>VS</td>
</tr>
<tr>
<td>Caren</td>
<td>Medium</td>
<td>2.08 a S 1.70 a S</td>
<td>MT</td>
<td>1.00</td>
</tr>
<tr>
<td>Hoëvelder</td>
<td>Long</td>
<td>1.95 a S 1.90 a S</td>
<td>MT</td>
<td>1.00</td>
</tr>
<tr>
<td>Aviva</td>
<td>Short</td>
<td>1.75 a S 2.10 a S</td>
<td>T</td>
<td>1.50</td>
</tr>
<tr>
<td>BP1</td>
<td>Medium</td>
<td>1.93 a S 2.20 ab S</td>
<td>VS</td>
<td>1.50</td>
</tr>
<tr>
<td>Bravo</td>
<td>Long</td>
<td>2.17 ab S 2.18 ab S</td>
<td>MT</td>
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<tr>
<td>Calibra</td>
<td>Medium</td>
<td>3.20 b VS 2.98 b S</td>
<td>T</td>
<td>2.00</td>
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<tr>
<td>Devlin</td>
<td>Short</td>
<td>2.75 ab VS 4.03 c VS</td>
<td>MT</td>
<td>1.75</td>
</tr>
<tr>
<td>Dawn</td>
<td>Medium</td>
<td>3.53 b VS 3.53 bc VS</td>
<td>S</td>
<td>1.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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*≤0.01; LSD=1.042 Spring 2000; LSD=0.867 Autumn 2001); S = susceptible, VS = very susceptible.

<sup>b</sup> Adapted from Nortje *et al.* (2000).

<sup>c</sup> MT = moderately tolerant, S = susceptible, T = tolerant, VS = very susceptible.

<sup>d</sup> 0 = no visible leaf infection, 4 = > 75% of foliage affected.

<sup>e</sup> 0 = no visible stem or leaf infection, 4 = > 75% of foliage affected.
APPENDIX H

EVALUATION OF CULTIVARS FOR SUSCEPTIBILITY TO VERTICILLIUM WILT

OPSOMMING

Die ontwikkeling van geneties stabiele, weerstandbiedende of tolerante kultivars word as die mees effektiewe benadering beskou vir die beheer van Verticillium-verwelk op aartappels. Tien aartappel-kultivars wat plaaslik verbou word, is oor drie seisoene in 'n glashuis geëvalueer vir bestandheid teen V. dahliae. Die kultivars Evan, Eryn, Hertha, Mnandi, Mondial en Ronn is as gematig vatbaar geklassifiseer, terwyl die kultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell en Up-to-date as vatbaar geklassifiseer is. Dit is belangrik om te onthou dat hierdie resultate afkomstig is vanaf glashuisproewe. Finale seleksie sal afhang van veldproewe waar die totale kompleks van agronomiese eienskappe soos opbrengs, knolgrootte verspreiding en knolvoorkoms, ook geëvalueer moet word.

SUMMARY

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 worldwide. Ten local potato cultivars were evaluated for resistance to Verticillium wilt over 3 seasons. The cultivars Evan, Eryn, Hertha, Mnandi, Mondial and Ronn were classified as moderate susceptible, while the cultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell and Up-to-date, were classified as susceptible to Verticillium wilt. It is important to keep in mind that these results are based on greenhouse trials. Final selection would depend on field trials in which the entire complex of agronomic traits such as yield, distribution of tuber size and tuber appearance, can be evaluated simultaneously.

INTRODUCTION

Developing genetically stable resistant or tolerant cultivars is the most efficient, economical, and environmentally sound approach to control Verticillium wilt in potato. Plant resistance to Verticillium wilt is defined as inability of the pathogen to penetrate the roots, interruption in host tissue colonization, 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, but apparently does not exist in potato. Certain potato varieties, however, show delayed or reduced colonization 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.

Traditional screening for resistance to Verticillium wilt involves growing potential germplasm in infested soil. 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. 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.
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. 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, Calibra, Caren, Darius, Eryn, Esco, Evan, Mnandi and Ronn, as well as 3 locally available foreign cultivars, Hertha, Mondial and Pentland Dell, were compared with the established cultivars, BP1 and Up-to-date, over three seasons from 2003 to 2005. Fifteen minitubers of each cultivar were planted in a greenhouse in a sterile mixture of sandy soil and vermiculite, artificially inoculated with microsclerotia of *V. dahliae*, in 15 cm pots. Fifteen pots without *V. dahliae* inoculum, per cultivar served as control. 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:
- 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. Plates were incubated at 25 °C for 6 days and examined microscopically for the presence of *V. dahliae*.

A disease index value based on the incidence and severity of visual wilting and yellowing symptoms, as well as stem colonization of the pathogen, was calculated. Based on differences in index values in infested soil and the corresponding control treatment, the cultivars were classified as follows:
- \( \leq 1.24 \) = resistant / tolerant
- \( \leq 2.49 \) = moderate susceptible
- \( \leq 3.74 \) = susceptible
- \( \geq 3.75 \) = very susceptible

**RESULTS**

The cultivars was classified into different reaction categories based on the incidence and severity of visual wilting and yellowing symptoms, as well as stem colonization of the pathogen. Results of the cultivars tested over three seasons from 2003 to 2005, are presented in Table 1 and Fig 1.

**CONCLUSION**

After a minimum of three seasons, the cultivars Eryn, Evan, Hertha, Mondial, Mnandi and Ronn were classified as moderate susceptible, while the cultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell
and Up-to-date, were classified as susceptible to *Verticillium* wilt. Plant resistance to *Verticillium* wilt is defined as inability of the pathogen to penetrate the roots, interruption in host tissue colonization, inactivation of toxic elements, or suppression of fungal sporulation. Disease tolerance is a form of resistance in which the plant becomes infected but goes on to mature and yields normally. Infected plants of the cultivars Eryn, Mnandi and Hertha were colonized with the pathogen, but did not differ significantly visually from their uninfected control treatments, during the Spring 2004 season. Therefore, they were classified as tolerant and not resistant during that specific season. Due to instability regarding reaction to *Verticillium* wilt, it is recommended that cultivars must be tested over 5 seasons rather than 3 seasons, before a final classification can be done. Furthermore, it is important to keep in mind that these results are based on greenhouse trials. Final selection would depend on field trials in which the entire complex of agronomic traits such as yield, distribution of tuber size and tuber appearance, can be evaluated simultaneously.

Table 1. *Verticillium* wilt reaction of selected locally produced cultivars after 3 seasons.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibra</td>
<td>3.2</td>
<td>2.98</td>
<td>2.38</td>
<td>3.03</td>
<td>4.46</td>
<td>VS</td>
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<td>1.7</td>
<td>2.12</td>
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<td></td>
<td>2.68 S</td>
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<tr>
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<td>1.93</td>
<td>2.2</td>
<td>1.47</td>
<td>4.41</td>
<td>VS</td>
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<td>Esco</td>
<td></td>
<td>3.09</td>
<td>2.12</td>
<td>4.67</td>
<td>VS</td>
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<td>3.29 S</td>
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<td>Up-to-date</td>
<td>3.47</td>
<td>3.19</td>
<td>2.52</td>
<td>S</td>
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<td>3.06 S</td>
</tr>
<tr>
<td>Pentland Dell</td>
<td>3.32</td>
<td>3.24</td>
<td>1.64</td>
<td>M</td>
<td></td>
<td></td>
<td>2.73 S</td>
</tr>
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<td>Darius</td>
<td>3.32</td>
<td>2.11</td>
<td>2.58</td>
<td>S</td>
<td></td>
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<td>2.67 S</td>
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<td></td>
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<td>3.98</td>
<td>VS</td>
<td>1.66</td>
<td>M 2.22 M</td>
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<td>VS</td>
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<td>R 2.13 M</td>
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<td>1.73</td>
<td>M</td>
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<td>1.95 M</td>
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<td>1.97</td>
<td>1.35</td>
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<td>1.77</td>
<td>1.16</td>
<td>1.88</td>
<td>M</td>
<td></td>
<td></td>
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<td>Hertha</td>
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<td>1.1</td>
<td>1.72</td>
<td>M</td>
<td></td>
<td></td>
<td>1.49 M</td>
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</table>
Fig. 1. *Verticillium* wilt reaction of selected locally produced cultivars after 3 seasons.
OPSOMMENDE VERSLAG

Inleiding
Verticillium-verwelk op aartappels is wêreldwyd 'n probleem en word in ander lande as selfs 'n groter probleem as Fusarium-verwelk beskou. Inteenstelling met Fusarium-verwelk wat relatief algemeen in Suid-Afrika op aartappels voorkom, is Verticillium-verwelk die eerste keer in Suid-Afrika gerapporteer in 1953, maar geen nuwe gevalle is daarna aangemeld vir ongeveer 40 jaar nie. 'n Moontlike verklaring is die ooreenkoms van die kenmerkende vergeling en verdroging simptome van die siekte met normale afsterwing simptome. Verder word vroeëre afsterwing ook toegeskryf aan onvoldoende voeding en besproeiing, onkruiddoderskade en ander siektes, veral Fusarium-verwelk. Sedert 1989 is vroeëre afsterwing van plante wat nie toegeskryf kon word aan enige fisiologiese skade of infeksie deur Fusarium nie, toenemend in sekere aartappel-produkserende areas waargeneem. Gedurende 1995 is daar met 'n opname begin om vas te stel of die siekte nie meer algemeen op aartappels voorkom nie. Na aanleiding van die resultate van die opname is 'n beheerstrategie vir die siekte voorgestel.

Opname
Alle plante met tipiese vergeling en verwelking simptome wat deur die diagnostiese sentrum te LNR-Roodeplaat gedurende die tydperk 1995 tot 2000 ontvang is, is getoets vir die teenwoordigheid van Verticillium-verwelk (Appendix A). Drie-en-negentig Verticillium isolate is vanuit 146 plantmonsters afkomstig vanaf 13 van die 14 aartappel-produkserende streke in die land geïsoleer. Sestig persent van die isolate is as Verticillium dahliae en 8% as V. nigrescens geïdentifiseer. Dertig van die isolate kon nie geïdentifiseer word nie. Meer as die helfte van die Verticillium isolate is vanuit die Sandveld geïsoleer. In vivo toetses het getoon dat beide V. dahliae en V. nigrescens patogenies is op aartappels, met V. dahliae die virulentste van die twee spesies.

Die tipiese simptome van Verticillium-verwelk op aartappels is vergeling en verwelking van die onderste blare gevolg deur verbruining en verdroging daarvan, waarna die simptome na die res van die stingel of selfs die hele plant, versprei (Fig. 1). Afsterwing van die plante kan al vanaf blomvorming voorkom, wat aanleiding gee tot die naam 'early dying disease'. Indien verwelkte stingels deursny word kan vaatbundel verkleuring waargeneem word (Fig. 2). Knolle word ook geïnfekteer en vaatbundel verbruining kan ook in die knolle voorkom (Fig. 3).

In 'n studie wat uitgevoer is deur LNR-Roodeplaat is 30 plante met tipiese vergeling en verwelking simptome, afkomstig vanaf dieselfde perseel, gebruik om te bepaal wat die verband tussen die voorkoms van visuele simptome (vergeling en verwelking van blare en vaatbundel-verbruining in stingels) en kolonisasie van vaatweefsel van stingels met die patogene is.
Fig. 1. Tipiese vergeling en verwelking simptome van Verticillium-verwelk van aartappels.

Fig. 2. Vaatbundelverbruining van aartappelstingels as gevolg van Verticillium-verwelk.
Fig. 3. Vaatbundelverbruining van aartappelknolle as gevolg van Verticillium-verwelk.

(Appendix I). Daar is gevind dat 50% van die plante met tipiese vergeling en verwelking simptome geen vaatbundel-verbruining simptome getoon het nie. Die feit dat 80% van die plante sonder vaatbundel-verbruining simptome, positief met V. dahliae gekoloniseer was, is egter kommerwekkend. Sestig % van die plante met vaatbundel-verbruining was positief vir V. dahliae (Fusarium oxysporum - verantwoordelike organisme van Fusarium-verwelk, was verantwoordelik vir die vaatbundel-verbruining simptome van die res van die plante). In dieselfde studie is ook bepaal in hoeveel gevalle is die nageslagknolle van ’n geïnfekteerde plant ook besmet. Daar is vasgestel dat daar gemiddeld ’n 53% kans is dat die nageslagknolle van ’n geïnfekteerde plant ook geïnfekteer sal word.

Alle plante met tipiese verwelking en vergeling simptome (selfs die sonder vaatbundel-verbruining) moet dus as potensiële gevalle van Verticillium-verwelk beskou word. Aangesien die afwesigheid van vaatbundel-verbruining in stingels en knolle nie noodwendig beteken dat die siekte nie voorkom nie, beteken dat die siekte produsente stilweg kan bekruip en onkant vang. Ons kan ook die gevolgtrekking maak dat die siekte wyer voorkom as wat die opname aangedui het.

**Beheerstrategie**

Die feit dat V. dahliae as hoof veroorsakende organisme van Verticillium-verwelk op aartappels in Suid-Afrika geïdentifiseer is, is slegs nuus vir produsente. Ten spyte van die potensiaal van geïnfekteerde saad as inokulumbron, kan V. dahliae ook op die wortels van plantegroei wat natuurlik in die omgewing voorkom, oorleef. V. dahliae kan vir tot 10 jaar dormant in die vorm van mikrosklerotia (Fig. 4) vry of geassosieerd met debrimateriaal, in grond oorleef. Indien die aantal mikrosklerotia in gronde ’n secere drumpelwaarde bereik, ontwikkel siekte waarvan die effek vererger word onder sekere strestoestande soos temperatuur en die hoeveelheid vog beskikbaar. Glaswaaier- en veldproewe het aangedui dat die drumpelwaarde so laag as 0.5 mikrosklerotia per gram grond kan wees (Appendix C). Indien aartappels herhaaldelik op dieselfde lande verteer word, kan die aantal mikrosklerotia in die grond drasties toeneem, aangesien die lewensiklus van die patogeen parallel met die groei en ontwikkeling van die aartappelplant verloop. Na planting stimuleer wortel eksudate van die ontwikkelende aartappelplant mikrosklerotia in die grond om te ontkiem. Tydens knolinisiasie penetreer hifes van die patogeen die plant wortels en beweeg tot in die vaatweefsel waar effektiewe vervoer van water en voedingstowwe verhinder word. Dit verklaar die tipiese vergeling en verwelking simptome van Verticillium-verwelk. Afsterwing van die plante kan vanaf blomvorming voorkom, wat aanleiding gee tot die naam “early dying disease”. Na loofafsterwing, vorm mikrosklerotia op die dooie stingels.
Tydens die oesproses word die stingels opgebreek in kleiner dele en in die grond geïnkorporeer. Op die wyse kan die patogeen dormant as mikrosklerotia in die grond oorleef.

![Fig. 4. Mikrosklerotia van *Verticillium dahliae*](image)

Beheer van die siekte berus op die verlaging van die aantal mikrosklerotia in die grond tot vlakke te laag vir siekte ontwikkeling op vatbare kultivars. As gevolg van beperkings op die gebruik van metielbromied as ‘n grondbehandeling, en kostes van berokingsmiddels soos metamsodium, word daar wêreldwyd meer gefokus op geïntegreerde beheermaatreëls, waar daar van meer as een praktik gebruik gemaak word om die siekte te beheer. Die verskillende beheermaatreëls moet sowel voor as na plant van die gewas geïmplementeer word. Die verschillende praktike wat gevolg moet word, kan aan die hand van ‘n schematische voorstelling soos volg verduidelik word (Fig. 5):

1) **Voor plant**

A) **Saadkeuse**

*Verticillium dahliae* is as dormante miselium teenwoordig in die vaatweefsel van geïnfekteerde knolle. Dit is belangrik om *V. dahliae* deur middel van ‘n vinnige, sensitiwe opsporingsmetode in ‘n saad sertifiseringsskema op te spoor, aangesien infeksie van die nageslagknolle kan plaasvind sonder dat daar simptoomuitdrukking plaasgevind het. Kommersieël beskikbare (DAS)-ELISA pakette vir die opsporing van *V. dahliae*, is in vitro geëvalueer (Appendix B). Die pakette word tans deur Coen Bezuidenhout Saadtoetssentrum as rutine toets vir die opsporing van *V. dahliae* uit nageslagknolle, in die saadsertifiseringsskema geëvalueer. Indien *Verticillium*-verwelk tydens die groeiseisoen voorgekom het, sal die beste besluit wees om die aartappels eerder op die tafelmark te
Fig 5. Geïntegreerde beheerstrategie vir die beheer van Verticillium-verwelk van aartappels.

verkoop as om dit as moere te hou, aangesien daar ten alle koste vermy moet word om geïnfekteerde moere te plant.

B) **Landkeuse**
Beheer van die siekte berus op die verlaging van die aantal mikrosklerotia in die grond tot vlakke te laag vir siekte ontwikkeling op vatbare kultivars. Dit is dus belangrik om die aantal lewensvatbare *V. dahliae* mikrosklerotia in grond te bepaal. Sodoende kan 'n siekte risiko bepaling van moontlike persele uitgevoer word, sowel as die effektiwiteit van beheermaatreëls geëvalueer word.

C) **Wisselbou**
Dit is baie belangrik dat wisselbougewasse wat in 'n rotasieprogram saam met aartappels verbou word, noukeurig gekies moet word aangesien *V. dahliae* 'n baie wye gashoedreeks het (Tabel 1). Die keuse van wisselbougewasse is verder ook belangrik in die toepassing van biobering. Bioberoking behels die plant van koolgewasse as wisselbougewas en die inwerk daarvan as groenbemesting ongeveer 2 weke voor die volgende gewas geplant word. Glukosinolaat, die karakteristieke swaelbevattende bestandeel van lede van die Crucifereae
word tydens die afbreekproses afgebreek na onder andere alliel isothiosianaat (AITC). AITC is ’n vlugtige stof wat net so toksies is vir swamme as metielisothiosianaat (MITC), die aktiewe bestandeel van kommersiële beskikbare grondberokingsmiddels. Biobowering is alreeds suksesvol met die wisselbou van kool as groenbemestingsgewas met aartappels vir die beheer van bruinskurf in Suid-Afrika bewys. Wisselbou met die broccoli as groenbemestingsgewas is suksesvol gebruik vir die beheer van *Verticillium*-verwelk van blomkool in die Salinas-vallei in Kalifornië. Vars en droë broccoli residue wat in 'n glashuisproef in kunsmatig geïnfesteerde grond ingewerk is, het die aantal lewensvatbare mikrosklerotia in grond, sowel as die ontwikkeling van die siekte in aartappelplante, betekenisvol verlaag (Appendix E).

Aangesien beheer van *Verticillium*-verwelk berus op die verlaging van *V. dahliae* mikrosklerotia in grond tot vlakke onder die drumpelwaarde vir siekteontwikkeling is die wisselbouperiode baie belangrik. ’n Wisselbou van twee tot drie jaar gaan nie werklik die inokulumvlakke in die grond verlaag nie aangesien *V. dahliae* mikrosklerotia as oorlewingstrukture vorm, en langer rotasieperiodes is dus nodig. Verder kan posisionering van gewasse soos peulplante, mielies, broccoli en braaklê die mate van sukses van die praktik tussen opeenvolgende aartappelaanplantings bepaal. Ongelukkig is die keuse van ’n wisselboustelsel nie altyd so eenvoudig nie en is verdere navorsing veral in die geval van *Verticillium*-verwelk nodig. ’n Veldproef om die effektiviteit van broccoli en kool as wisselbougewasse in ’n 3 jaar-wisselbouprogram vir die beheer van die siekte te bepaal, is begin gedurende September 2003 in die Gamtoos-vallei in die Oos-Kaap. As gevolg van die teenwoordigheid van die Goue sistaalwurm, is die proef getermerne.

### Tabel 1. Gasheerreeks van *Verticillium dahliae*.

<table>
<thead>
<tr>
<th>Groente</th>
<th>Ander gewasse</th>
<th>Bome en blomme</th>
<th>Onkruide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aartappels</td>
<td>Aarbeie</td>
<td>Appelkose</td>
<td>Akkererepys</td>
</tr>
<tr>
<td>Artisjokke</td>
<td>Grondboontjies</td>
<td>Avokado</td>
<td>Akkerwinde</td>
</tr>
<tr>
<td>Beet</td>
<td>Katoen</td>
<td>Kersie</td>
<td>Blouseblommetjie</td>
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<tr>
<td>Blomkool</td>
<td>Lusern</td>
<td>Olyf</td>
<td>Bitterappel</td>
</tr>
<tr>
<td>Brusselse spruite</td>
<td>Okra</td>
<td>Pistachio</td>
<td>Doring boetebossie</td>
</tr>
<tr>
<td>Chinese Kool</td>
<td>Satfloer</td>
<td>Antirrhinun</td>
<td>Dubbeltjie</td>
</tr>
<tr>
<td>Eiervrug</td>
<td>Sojabone</td>
<td>Rose</td>
<td>Geeluintjie</td>
</tr>
<tr>
<td>Kool</td>
<td>Sonneblomme</td>
<td>Groot boetebossie</td>
<td>Porslein</td>
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<td>Koolrape</td>
<td>Spanspek</td>
<td>Herderstassie</td>
<td>Purperwind</td>
</tr>
<tr>
<td>Komkommer</td>
<td>Tabak</td>
<td>Indringer-Ageratum</td>
<td>Satansbos</td>
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<td>Pampeoen</td>
<td>Waatlemoen</td>
<td>Kakiebos</td>
<td>Sida</td>
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<tr>
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<td>Kanadese skraalh</td>
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<td>Kankerroos</td>
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<td>Kleinkakiebos</td>
<td>Terblansbossie</td>
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<td>Klimop</td>
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<td>Kilstklawer</td>
<td>Wandelingen jood</td>
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<td>Rissies</td>
<td>Knapskêrel</td>
<td>Wiblombloedissel</td>
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<tr>
<td>Seldery</td>
<td>Knopkruid</td>
<td>Wit hondebossie</td>
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<tr>
<td>Soetrisse</td>
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<td>Suikerbeet</td>
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<td>Tamaties</td>
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</tbody>
</table>
D) Sanitasie

Om te voorkom dat die patogeen dormant as mikrosklerotia in die grond oorleef, moet daar so vinnig as moontlik van dooie plantreste ontslae word deur dit te brand of diep in te ploeg (Appendix D). Verder is die sanitasie van kratte, sorteertafels en koelkamers belangrik. Dit is ook uiteres belangrik dat implemente en skoene ook ontsmet moet word, aangesien grondpartikels aan implemente en skoene vassit en op die wyse kan die siekte maklik van 'n land na 'n ander oorgedra word. Verskeie middels is getoets en kan met sukses gebruik word vir die ontsmetting van implemente, koelkamers, kratte, vloeroppervlaktes en sorteertafels (bv. kalsiumhipochloried, natriumhipochloried en karbolsuur).

E) Bewerking

Aangesien die mikrosklerotia van *V. dahliae* hoofsaaklik in die boonste 20-30 cm van die grond voorkom, kan die boonste grondlaag met bewerkingstrukture soos diepploeg, dieper in die grond in geploeg (begrawe) word en skoner grond na bo gebring word. Die patogeen word dus uit die wortelsone waar infeksie plaasvind verwyder. Die sukses van die praktyk lê in die effektiewe draai van die boonste laag sodat “skoner” grond na bo kan kom. Geen sukses gaan behaal word indien die grond net deurgemeng word nie. Grondtype speel egter ‘n belangrike rol in die suksesvolle toepassing van die tegniek. Tydens ’n eksperiment op Roodeplaat waar ’n gewone skaarbewerking vergelyk is met geen bewerkingstrukture (in ’n Hut tengrondtype) is ’n siekte soos antraknose (wat deur *Colletotrichum coccodes* veroorsaak word) suksesvol verlaag. Die metode kan dus moontlik ook suksesvol toegepas word vir die beheer van *V. dahliae* wat net soos *C. coccodes* ook mikrosklerotia as oorlewingstrukture vorm.

F) Beheerpraktekies

Chemies

Alhoewel grondinokulum van *V. dahliae* chemies beheer kan word, is chemiese beheer ‘n baie duur praktyk en is die effektiwiteit van beheer ook gekoppel aan die aanvanklike inokulumvlakke in die grond. Chemiese produkte kan dus net ‘n siekte-onderdrukkende effek toon waar die aantal mikrosklerotia in die grond deur die produk verlaag word, maar nie tot onder die drumpelwaarde wat vir siekte-ontwikkeling nodig is nie. As gevolg van beperkings op die gebruik van metilbromide as grondbehandeling, en kostes van berokingsmiddels soos metamsodium en chloropikrien, word daar egter wêreldwyd meer gefokus op geïntegreerde beheermaatreëls, waar daar van meer as een omgewings-vriendelike praktyke gebruik gemaak word om die siekte te beheer.

Solarisering

Dit is ‘n praktyk waar plastiek vir ‘n tydperk oor ‘n land getrek word, met ‘n gevolglike styging in grondtemperatuur wat sodoende die siekte kan beheer. Die tegniek word suksesvol in lande soos Israel gebruik vir die beheer van die siekte. Die sukses van die tegniek word bepaal deur die omgewingstoestande en kan dus nie uitgevoer word tydens die koeler maande van die jaar nie aangesien hoër temperatuur ‘n sleutelfaktor is. Tydens ‘n eksperiment op Roodeplaat is solarisering as effektief vir die beheer van antraknose bepaal. Die metode kan ook suksesvol toegepas word vir die beheer van *V. dahliae* wat net soos *C. coccodes* ook mikrosklerotia as oorlewingstrukture vorm.

Pes en siekte beheer

Die effektiewe beheer van knolgedraagde siektes soos nematodes, *Rhizoctonia solani* (swartskurf) en *Colletotrichum coccodes* (antraknose) is baie belangrik. Hierdie siektes kan groeistremming op die plant plaas wat die plant meer vatbaar vir infeksie van *V. dahliae* maak. In die ‘early dying disease’ kompleks is *V. dahliae* sowel as nematodes die veroorsakende organisme. Daar bestaan nog twyfel oor die spesifieke mecanisme wat hier betrokke is, maar basies kom dit daarop neer dat wanneer die twee organisme saam...
voorkom, die effek daarvan soveel groter is as wanneer die twee alleen voorkom. Daar bestaan tans nie plaaslike data in verband met hierdie verskynsel nie.

**Onkruidbeheer**

Goeie onkruidbeheer is baie belangrik aangesien ‘n glashuisproef waar verskeie onkruide wat algemeen op aartappelveld oorkom in kunsmatige geïnfecteerde grond geplant is, aangedui het dat onkruide soos kakkiebos, nastergal, wandelende jood, suring en sydissel gashere van die patogeen is (Appendix F). Verder kan mikrosklerotia ook saprofities op dooie onkruid soos geeluintjies, klimop, knopkruid en mexikaanse papawers oorleef. ‘n Lys van onkruide wat as alternatiewe gashere van die patogeen kan dien word in Tabel 1 aangedui.

**G) Kultivarkeuse**

Die ontwikkeling van geneties stabiele, weerstandbiedende of tolerante kultivars word as die mees effektiewe benadering beskou vir die beheer van *Verticillium*-verwelk op aartappels. Tien Suid-Afrikaanse aartappelkultivars, waarvan agt onlangs vrygestel is, is oor twee seisoene gedurende 2000 en 2001 in ‘n glashuis geëvalueer vir bestandheid teen *V. dahliae* (Appendix G). Die kultivars Aviva, BP1, Bravo, Buffelspoort, Caren, Hoëvelder en Ropedi is as vatbaar vir *Verticillium*-verwelk geklassifiseer, terwyl Calibra, Dawn en Devlin geel oorleef. Dertien aartappelkultivars wat plaaslik geïnfecteer is, is oor drie seisoene gedurende 2003 tot 2005 in ‘n glashuis geëvalueer vir bestandheid teen *V. dahliae*. Die kultivars Evan, Eryn, Hertha, Mnandi, Mondial en Ronn is as gematig vatbaar geklassifiseer, terwyl die kultivars BP1, Calibra, Caren, Darius, Esco, Pentland Dell en Up-to-date as vatbaar geklassifiseer is (Appendix H). Dit is belangrik om te onthou dat hierdie resultate afkomstig is vanaf glashuisproewe. Fele seleksie sal afhang van veldproewe waar die totale kompleks van agronomiese eienskappe soos opbrengs, knolgrootte verspreiding en knolvoorkoms, ook geëvalueer moet word.

**H) Plantdatum**

Die keuse van ‘n plantdatum kan ook ‘n belangrike rol speel ten einde moontlike stremmingstoestande soos die groei l dés loeng te probeer vermy. Aartappels groei optimaal by 18–20°C, terwyl die optimale temperatuur van *V. dahliae* tussen 22–27°C is. ’n Styging in die gemiddelde lugtemperature van 20-28°C is dus bevorderlik vir siekteontwikkeling.

2)  

**Na plant**

**A) Sanitasie**

Soos voorheen gemeld is sanitasie van implemente en spuittoerusting wat tydens die groeiseisoen gebruik word van kardinale belang om die oordraging van die patogeen na siekte-vrede lande te verhoed

**B) Bemesting**

Ten einde ‘n goeie opbrengs te behaal, is ‘n grondontleding van die land van kardinale belang om die regte hoeveelheid voedingselemente te gee. Dit geld ook vir *Verticillium*-verwelk want, indien voedingstekort in die groeiseisoen voorkom, gaan stremming op die plant die kans op infeksie en siekte-ontwikkeling bevorder. Die grondontleding word as basis gebruik vir ‘n bemestingsprogram om optimale groei te handhaaf.

**C) Besproeiingskedulering**

Oorbesproeiing vroeg in die groeiseisoen is bevorderlik vir siekte-ontwikkeling en onderbesproeiing later in die groeiseisoen is bevorderlik vir simptoomuitdrukking van *Verticillium*-verwelk. Besproei dus optimaal om groeistremming op die plant te vermy en die kans op infeksie te verlaag. Verder is besproeiing met
brakwater ook bevorderlik vir siekte-ontwikkeling. Plaaslike data i.v.m die invloed van besproeiing op die voorkoms van die siekte is nie beskikbaar nie.

D) Beheerpraktyke
Alle vorme van siektebeheer op die loof, veral vroeë- en laatroes is belangrik. Vermy dus alle vorme van stremming wat optimale groei belemmer.

E) Probleemlande
Dit is van kardinale belang dat, waar ‘n siekte soos Verticillium-verwelk op ‘n land geïdentifiseer is, daar ‘n aantekening gemaak moet word vir toekomstige aanplantings. Dit kan so maklik gebeur dat na ‘n paar seisoene van bv. mielieverbouing sulke probleme vergete raak. Hou dus rekord van alle siektes wat op ‘n betrokke land voorgekom het.

‘n Effektiewe beheer strategie vir die beheer van Verticillium-verwelk van aartappels behels ‘n gestrukureerde, goed beplande strategie wat die gebruik van verskeie metodes wat op verskillende tye gedurende die groeiseisoen, en geïntegreerd in die totale gewasproduksie sisteem, toegespits word. ‘n Beheerstrategie wat op die gebruik van verskeie metodes berus is meer volhoubaar en effektief as een wat op ‘n enkele metode berus.

Punte wat nog verder aangespreek kan word:
1. Effektiwiteit van bioberoking vir die beheer van Verticillium-verwelk – veldproef.
2. Evaluering van teellyne vir bestandheid teen Verticillium-verwelk.
3. Effektiwiteit van PCR vir die opsporing van V. dahliae in plantmateriaal.
4. Verwantskap tussen inokulumdigtheid van V. dahliae en nematode-tellings in grond m.b.t simptome-uitdrukking.
5. Invloed van besproeiing en bemesting op die voorkoms van Verticillium-verwelk.
6. Effektiwiteit van organiese byvoegings vir die beheer van Verticillium-verwelk.