Reproduction and Damage Potential of Five Geographical Ditylenchus africanus Populations on Peanut

SONIA STEENKAMP,1 DIRK DE WAEL,2,3 AND ALEXANDER MCDONALD3

Abstract: Ditylenchus africanus affects peanut quality, which leads to downgrading of consignments and economic losses for producers. This nematode is difficult to control and host-plant resistance may be the most effective way to control it. Recently, the peanut breeding line PC254K1 has been identified as resistant to a D. africanus population from Vaalharts and will be included into the peanut breeding program. The objectives of our study were to compare the reproduction potential of D. africanus geographic populations from five different areas in the peanut production area of South Africa and to assess whether PC254K1 is resistant to all five D. africanus populations. Reproduction of the D. africanus populations was evaluated on peanut callus in growth cabinets at 21°C, 28°C, and 35°C. The peanut cv. Selfie was included in the study as the D. africanus-susceptible reference genotype in the greenhouse and microplots. Reproduction potential of all five of the D. africanus populations was similar. Resistance of PC254K1 was confirmed to all five D. africanus populations. The resistance trait of a D. africanus-resistant cultivar developed from PC254K1 should, therefore, be sustainable over the five localities tested during this study.

Key words: Arachis hypogaea, Ditylenchus africanus, peanut, pod nematode, resistance.

Ditylenchus africanus Wendt, Swart, Vrain & Webster, the peanut pod nematode, is omnipresent in all peanut production areas of South Africa (De Waele et al., 1989). All indications are that this nematode species is endemic to the country as there are no confirmed reports of its presence outside South Africa (Dickson and De Waele, 2005). This migratory endoparasitic nematode can infect various agricultural crops (Basson et al., 1990) and weeds (De Waele et al., 1990) but causes damage only to peanut (De Waele et al., 1989). It is considered to be one of the economically most important pathogens that limit peanut production in South Africa (Venter et al., 1991). Ditylenchus africanus enters the pod at the connection point between the pod and the peg (De Waele et al., 1989), which causes the peg and pod connection to weaken so that the pods break off during lifting of the plants at harvest (Jones and De Waele, 1990). In heavily infested fields, D. africanus may cause losses of 40% to 60% of the pods in such a way (Jones and De Waele, 1988). The main effect of D. africanus on peanut consignments, however, is qualitative (Mc Donald et al., 2005) causing downgrading of the unattractive, infected seeds of peanut consignments to lower grades (Jones and De Waele, 1990). Damage caused by D. africanus to the hull of the pod allows water to enter the pod (Venter et al., 1995) and weakened pods often split open (De Waele et al., 1997). The deteriorated and split hulls then result in the germination of second-generation seedlings (Venter et al., 1995; De Waele et al., 1997). Ditylenchus africanus also feeds on the seed testa (Jones and De Waele, 1990), causing chemical compounds that functions as inhibitors of seed germination to leach out (Svamv and Narasimhareddy, 1977), which also results in the growth initiation of the hypocotyls (De Waele et al., 1997). Feeding of the nematodes near or in the vascular bundles of the seed testa furthermore results in an unattractive appearance of infected seed (Jones and De Waele, 1990). The symptoms of D. africanus infection have a negative impact on the percentage of unsound (mold-infested kernels, kernels decayed, chalky, damaged by insects or heat or kernels that show internally or under the testa any discoloration not typical of sound kernels), blemished (whole kernels with colored streaks or blotches in or on the testa), and soiled (whole or split kernels soiled to such an extent that their appearance is affected) kernels (%UBS) (Government Gazette, 2005) causing downgrading of consignments (Mc Donald et al., 2005). Ditylenchus africanus infection, therefore, can have substantial financial implications for a producer (Van der Merwe and Joubert, 1992).

From an economic and environmental perspective, host plant resistance is one of the most preferred tools for the management of plant-parasitic nematodes on a variety of agricultural crops (Starr et al., 2002; Agudelo et al., 2005; Dickson and De Waele, 2005; Cook and Starr, 2006). This also applies to the management of D. africanus on peanut (De Waele et al., 1990). Recently, the breeding line PC254K1 was confirmed to be highly resistant to D. africanus (Steenkamp et al., 2010) and showed potential for inclusion in the local peanut breeding program as a primary source of resistance.

Acceptance of a new cultivar developed from PC254K1 by the farmers will not only depend on the agronomic acceptability of the new cultivar but also on the sustainability of its resistance to D. africanus. Sustainability of resistance to plant-parasitic nematodes will depend on the reproduction and damage potential (virulence) of the nematode populations present in

Received for publication December 1, 2015.

1Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom 2520, South Africa.
2Laboratory of Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), Willem de Croylaan 42, 3001 Heverlee, Belgium.
3Unit of Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa.

The authors thank L. Brinkhors, R. Jantjes, S. Kvena, A. Tladi, and B. Mathuli for technical assistance and the Oil and Protein Trust and ARC-Grain Crops Institute for financial support.

E-mail: SteenkampS@arc.agric.za.

This paper was edited by Richard Davis.
the fields in which the new cultivar is grown (Blok et al., 1997; Sree Latha et al., 1998; Noe, 1992; Thies and Ferry, 2002). Thus, differences in reproduction and damage potential of geographical plant-parasitic nematode populations may affect the efficacy of the nematodes’ management. Knowledge of differences in pathogenicity among *D. africanus* populations present in the various peanut production areas is, therefore, necessary for the successful development and use of resistant cultivars.

A number of studies have been carried out on the reproduction of *D. africanus* on peanut callus tissue (Van der Walt and De Waele, 1989) at temperatures that ranged from 16°C to 34°C (De Waele and Wilken, 1990) and on the reproduction and damage potential of *D. africanus* on a number of peanut genotypes (Basson et al., 1991, 1992, 1993; Venter et al., 1991, 1993; Van der Merwe & Joubert, 1992; McDonald et al., 2005). No comparison has been made so far of the reproduction and damage potential of *D. africanus* populations isolated from different geographical locations in South Africa, however. Therefore, the objective of our study was to establish whether there are differences in the reproduction and damage potential of *D. africanus* originating from different localities in the peanut-producing areas of South Africa.

**Materials and Methods**

*Ditylenchus africanus* populations: The *D. africanus* populations included in our study were originally isolated from infected peanut pods collected from infested fields in Mareetsane (26.15°S, 25.43°E), Jan Kempdorp (27.95°S, 24.85°E), Vaalharts (27.83°S, 24.79°E), Schweizer-Renecke (27.19°S, 25.33°E), and Theunissen (28.40°S, 26.71°E) (Fig. 1). The infected pods were collected from peanut shelling plants at the abovementioned locations by officers of the Perishable Products Export Control Board of South Africa. The material was delivered to the Nematology Unit of Agricultural Research Council-Grain Crops Institute in Potchefstroom within 2 to 3 d of collection. After collection, the pods were put in dry cooler bags and were stored at room temperature under air-dry conditions until extraction of the nematodes. Twenty pods from each locality were shelled by hand and on average

![Groundnut production areas](image)

Fig. 1. *Ditylenchus africanus* populations isolated from infected peanut pods at five different localities (red dots) within the peanut production area of South Africa.
251,484 *D. africanus* (a mixture of juveniles and adults) were extracted from the pod tissues (5 g hull + 5 g kernel) from each locality using the soaking method described by Bolton et al. (1990). In vitro aseptic callus tissue cultures were initiated from surface-sterilized leaves of the peanut cv. Sellie (De Waele and Wilken, 1990). From each of the abovementioned *D. africanus* populations, five females and five males were hand-picked, sterilized using 2% streptomycin sulfate and added on the callus tissues to establish the nematode cultures (Van der Walt and De Waele, 1989). The peanut callus tissues were grown in the dark on growth medium described by Van der Walt and De Waele (1989) in petri dishes placed in a growth cabinet at 26°C. *Ditylenchus africanus* obtained from these callus tissue cultures was then used as inoculum.

**Effect of temperature on different population reproduction:** Three growth cabinets were set at 21°C, 28°C, and 35°C, respectively, and allowed to stabilize for 3 d at the respective temperatures before proceeding with the trial. Ninety peanut calluses (cv. Sellie) of approximately 1.5 g each were used. Each of the 90 calluses was inoculated with five males and five females of the different *D. africanus* populations following the procedures described by Van der Walt and De Waele (1989).

The trial was laid out in split-plot completely randomized design with a factorial arrangement of treatments. The three different temperature regimes were the main factor. The callus tissue cultures of each of the five *D. africanus* populations, each replicated six times, were the subfactor. The inoculated calluses were incubated in the dark for 4 wk in the growth cabinets to allow the nematodes to complete a minimum of four life cycles (De Waele and Wilken, 1990). After this, the nematodes were extracted from the callus tissue as follows: Nematodes were recovered from the callus tissue by rinsing off the nematodes present on the lid of the petri dish and on the surface of the intact callus tissue and growth medium with a slow trickle of tap water. The callus tissue and the growth medium were then sectioned, placed on a 45-μm sieve, and rinsed with tap water. Nematodes were extracted from the callus tissue as described by De Waele and Wilken, 1990. The juveniles and adults from each population and the amount of kernels left for reliable yield assessments was insufficient.

**Effect of peanut cultivar on different population reproduction in microplots:** Sixty plots, 0.5-m deep with a diameter of 1 m, were filled with the same fumigated sandy-loam soil used in the greenhouse trial. Each plot was then planted with six seeds of either the cv. Sellie or the breeding line PC254K1 to a depth of 5 cm. Prior to planting, the seeds were treated with the fungicide Tiram (dithiocarbamate) at 120 g per 500 kg seed (Thiolin; Almond Agro Chemicals [Edms] Bpk, Potchefstroom, South Africa) and inoculated with *Bradyrhizobium arachis* nitroge-fixing bacteria at 250 g per 50 kg seed (Soygro [Edms] Bpk, Potchefstroom, South Africa). One seed per pot was planted to a depth of 5 cm.

The plants were placed in a split-plot completely randomized design in a greenhouse with a temperature regime of 18°C to 27°C with a 13-h photoperiod. Factor one was a plot of 30 pots of either the cv. Sellie (susceptible) or the breeding line PC254K1 (resistant), with six single-plant replicates inoculated with each of the five *D. africanus* populations. The two peanut genotype pots were grouped together and completely randomized within each group.

Each pot was inoculated at planting with ±2,000 *D. africanus* consisting of various life stages (juveniles and adults). Plants were inoculated by pipetting the required volume of nematode suspension evenly over the seeds. Plants were watered three times a week.

Nematode assessments were made at harvest on mature plants 20 wk after planting, using all the plants. *Ditylenchus africanus* was extracted from pegs as described by De Waele et al. (1987) and from hulls and kernels using the soaking method (Bolton et al., 1990). Nematodes were counted per 5 g pegs, 5 g pods, and 5 g kernels, added and expressed as Pf per 15 g pods. Yield assessments were not made from the single-plant pots in the greenhouse trial because the technique used for nematode extraction is destructive (Bolton et al., 1990) and the amount of kernels left for reliable yield assessments was insufficient.

**Effect of peanut cultivar on different population reproduction in the greenhouse:** Sixty 4,000-cm³ plastic pots were filled with an ethylene dibromide-fumigated (at an equivalent of 50 liter/ha, 3 wk before planting), sandy-loam, Hutton soil (93.6% sand, 3.9% clay, 1.9% silt, and 0.6% organic material, pH 6.28). Nutrients were added to the soil according to a soil analysis and nutrient guidelines for peanut (Swanevelder, 1997). Prior to planting, the seeds were treated with the fungicide Tiram (dithiocarbamate) at 120 g per 500 kg seed (Thiolin; Almond Agro Chemicals [Edms] Bpk, Potchefstroom, South Africa) and inoculated with *Bradyrhizobium arachis* nitroge-fixing bacteria at 250 g per 50 kg seed (Soygro [Edms] Bpk, Potchefstroom, South Africa). One seed per pot was planted to a depth of 5 cm.

The plants were placed in a split-plot completely randomized design in a greenhouse with a temperature regime of 18°C to 27°C with a 13-h photoperiod. Factor one was a plot of 30 pots of either the cv. Sellie (susceptible) or the breeding line PC254K1 (resistant), with six single-plant replicates inoculated with each of the five *D. africanus* populations. The two peanut genotype pots were grouped together and completely randomized within each group. Factor 2 consisted of the five geographical *D. africanus* populations. The inoculum density was ±2,000 *D. africanus* of various life stages per plant at planting, inoculated the same way as described for the greenhouse trial. Every treatment was replicated six times. Supplementary to the rainfall, plots were irrigated three times a week using a microsprayer placed in the center of each plot. Supplementary to rainfall,
plots were irrigated three times a week using a micro-sprayer placed in the center of each plot.

Nematode and damage assessments were done at harvest on mature plants, approximately 20 wk after planting. Separate nematode extractions were done from peg, hull, and kernel samples collected from two randomly picked plants from each plot. *Ditylenchus africanaus* was extracted, counted, and expressed the same way as done for the greenhouse trial.

The four remaining plants in each plot not used for nematode extraction were used for damage assessment. Yield quantity was not determined for this part of the study because *D. africanaus* does not affect overall yield (Venter et al., 1991, 1992; McDonald et al., 2005). Yield quality was determined by following standard grading procedures stipulated by the Act on Agricultural Product Standards, 119 of 1990 (South Africa, 1990).

**Statistical analyses:** Unless otherwise stated, nematode data were transformed by \(\ln(x + 1)\) before being subjected to a factorial analysis of variance (Stat Graphics 5 Plus for Windows). Means were separated by a least significant difference test \((P < 0.05)\). All differences are significant at \(P < 0.05\) unless otherwise stated.

Nematode variables included final population densities \((P_f)\) of *D. africanaus* present in callus tissue cultures in the growth cabinet trial and \(P_f\) present in pods of cv. Sellie and PC254K1 in the greenhouse and microplot trials. The reproduction factor \((RF)\) of *D. africanaus* on callus tissue cultures (growth cabinet trial) and pods of cv. Sellie and PC254K1 (greenhouse and microplot trials) was determined using Oosterbrink’s equation: 

\[
RF = \frac{P_f}{P_i \cdot \ln(x + 1)}
\]

where \(P_f\) is the population density of the callus tissue cultures \((\text{callus tissue cultures} (\text{growth cabinet trial}) \text{ and pods of cv. Sellie and PC254K1})\) and \(P_i\) is the initial population density. 

**RESULTS**

**Effect of temperature on different population reproduction:** There was no significant interaction between temperature regime and *D. africanaus* population densities \((P\text{-value}: 0.4969; \text{Fratio: 0.93})\). However, there were differences among the populations at 21°C as well as at 28°C (Table 1). At 21°C, the \(P_f\) of the Schweizer-Renecke population was greater than that of the Mareetsane, Jan Kempdorp, Vaalharts, and Theunissen populations (Table 1).

The \(P_f\) of the Mareetsane, Jan Kempdorp, Vaalharts, and Theunissen populations did not differ significantly from each other. At 28°C, the \(P_f\) of the Schweizer-Renecke population was higher than that of the Jan Kempdorp and Vaalharts populations (Table 1). No significant differences were observed among the \(P_f\) of the five populations at the 35°C regime (Table 1).

The \(P_f\) of all five populations at all three temperature regimes was greater than 1 (Table 1). The \(P_f\) of the populations at 28°C were 58 to 373 times greater than those of the same populations on callus tissues at 21°C and 35 to 156 times greater than those of the same populations at 35°C. Mean \(RF\) values at 35°C were only slightly greater than those at 21°C.

**Effect of peanut cultivar on different population reproduction in the greenhouse**

**Final nematode population densities and RF:** A significant interaction existed between the peanut genotypes and the \(P_f\) of the five *D. africanaus* populations in the greenhouse trial \((P\text{-value}: 0.0313; \text{Fratio: 2.92})\). The \(P_f\) of all five populations was greater on cv. Sellie than on PC254K1 (Table 3). No significant differences existed among the five populations on cv. Sellie or on PC254K1 (Table 3).

The \(RF\) of all five populations on cv. Sellie was greater than 1 (Table 3). In contrast, the \(RF\) on PC254K1 was all less than 1.

**Final nematode population densities and RF:** No significant interaction existed among the five populations and the two peanut genotypes \((P\text{-value}: 0.9658; \text{Fratio: 1})\).
Table 2. Final nematode numbers (Pf) on peanut callus tissue inoculated with five geographical Ditylenchus africanus populations pooled at three temperature regimes.

<table>
<thead>
<tr>
<th>Temperature regimes (°C)</th>
<th>Pf [ln(x + 1)]</th>
<th>RF</th>
<th>Pf [ln(x + 1)]</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>3.16 b</td>
<td>1.21</td>
<td>3.54 b</td>
<td>0.40</td>
</tr>
<tr>
<td>28</td>
<td>7.19 a</td>
<td>2.52</td>
<td>7.69</td>
<td>2.88</td>
</tr>
<tr>
<td>35</td>
<td>3.54 b</td>
<td>1.38</td>
<td>3.16 b</td>
<td>0.27</td>
</tr>
<tr>
<td>Fratio</td>
<td>76.69</td>
<td>4.28</td>
<td>3.25</td>
<td>1.09</td>
</tr>
<tr>
<td>P value</td>
<td>0.0000</td>
<td>0.05</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Numbers in the same column followed by the same letter do not differ significantly at P < 0.05.

Table 4. Final Ditylenchus africanus numbers (Pf) and reproduction factors (RF) of five geographical D. africanus populations in pods of cv. Sellie (susceptible) and PC254K1 (resistant) under microplot conditions at harvest.

<table>
<thead>
<tr>
<th>Population</th>
<th>Pf [ln(x + 1)]</th>
<th>RF</th>
<th>Pf [ln(x + 1)]</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mareetsane</td>
<td>6.29 a</td>
<td>0.51</td>
<td>3.55 b</td>
<td>0.08</td>
</tr>
<tr>
<td>Jan Kempdorp</td>
<td>6.21 a</td>
<td>0.27</td>
<td>3.85 b</td>
<td>0.03</td>
</tr>
<tr>
<td>Vaalharts</td>
<td>6.26 a</td>
<td>0.29</td>
<td>3.25 b</td>
<td>0.02</td>
</tr>
<tr>
<td>Schweizer-Renecke</td>
<td>6.88 a</td>
<td>0.68</td>
<td>4.19 b</td>
<td>0.03</td>
</tr>
<tr>
<td>Theunissen</td>
<td>6.19 a</td>
<td>0.25</td>
<td>3.13 b</td>
<td>0.02</td>
</tr>
<tr>
<td>Fratio</td>
<td>1.21</td>
<td>-</td>
<td>0.40</td>
<td>-</td>
</tr>
<tr>
<td>P value</td>
<td>0.3586</td>
<td>-</td>
<td>0.8087</td>
<td>-</td>
</tr>
</tbody>
</table>

a RF = Pf/Pi.
b Numbers in the same column followed by the same letter do not differ significantly at P < 0.05.

The low Pf of the five populations in the pods of cv. Sellie and PC254K1 were relatively low for all five populations (Table 4). Although the Pf of all the corresponding nematode populations were greater on cv. Sellie than on PC254K1, there were no significant differences among the respective populations for either genotype.

The low Pf of the five populations in the pods of cv. Sellie and PC254K1 were also reflected in their respective low RF, which remained less than 1 for both genotypes (Table 4).

Damage assessments: The percentage of unsound, blemished, and soiled kernels (%UBS) of PC254K1 varied and ranged from 4% (Vaalharts) to 9% (Schweizer-Renecke and Theunissen) (Fig. 2). PC254K1 produced choice grade (Vaalharts) or standard grade (Mareetsane, Jan Kempdorp, Schweizer-Renecke, and Theunissen). In contrast with the %UBS of PC254K1 kernels those of cv. Sellie were all greater than 10%, ranging from 12% to 14%. Sellie kernels graded crushing and diverse grade.

Discussion

No significant interaction between temperature regimes and Pf of the five different D. africanus populations in the growth cabinet trial implies that the increase in population at the different temperature regimes was similar for all populations. However, the data of this trial show that temperature regime has a significant effect on D. africanus population growth and that 28°C is close to the optimum temperature for the reproduction of this nematode species (De Waele and Wilken, 1990) for a variety of different populations. The lower Pf at 21°C and at 35°C compared to that of 28°C may be attributed to a reduction in egg production and development (De Waele and Wilken, 1990).

In the greenhouse trial, the D. africanus populations behaved differently on cv. Sellie than on PC254K1. The lack of a significant interaction between the two peanut genotypes and the five D. africanus populations implies that the increase of the D. africanus populations on each of the two peanut genotypes was similar. In contrast to the greenhouse trial, the five D. africanus populations did not behave differently in terms of reproduction rate on cv. Sellie and PC254K1 in the microplots since no significant interaction existed between the two peanut genotypes and the five populations. The lower nematode numbers on cv. Sellie in the microplot trial compared to the greenhouse trial have previously been reported (Venter et al., 1991; Mc Donald et al., 2005). However, the low Pf of the nematodes extracted from pods of PC254K1 in the greenhouse and microplot trial confirm PC254K1’s resistance to all five D. africanus populations. Resistance of a host plant can be determined by its effect on nematode reproduction (Trudgill, 1986).
That could be defined as the ability to inhibit the reproduction of a population relative to the reproduction of the population on a susceptible host (Cook and Evans, 1987; Roberts, 2002).

These data further indicate that all the *D. africanus* populations were still able to increase at 21°C and 35°C even though their reproduction rate was not optimal. The resistance of PC254K1 to *D. africanus* was again confirmed by RF in the greenhouse as well as in the microplots (Roberts and May, 1986; Windham and Williams, 1988).

The small differences in %UBS among the five different populations in cv. Sellie indicate that the damage potential of the populations on peanut is similar. Mc Donald et al. (2005) showed strong relationships between the presence of *D. africanus* and %UBS. Venter et al. (1991) also provided strong evidence that %UBS is a reliable indication of *D. africanus* damage. The low %UBS of PC254K1 compared to cv. Sellie is, therefore, a further confirmation of its resistance to all five *D. africanus* populations. The differences, though being small, among the %UBS of the populations do not agree with differences in Pf or RF of either cv. Sellie (susceptible) or PC254K1 (resistant). This part of the study, however, was not repeated and warrants further research.

The results of our study indicated that the reproduction and damage potential of the five *D. africanus* populations representative of the local peanut production area were similar. The near optimal temperature for the development of *D. africanus* populations is 28°C, but they are able to reproduce (although not optimally) over a wide range of temperatures. Resistance of PC254K1 to all five *D. africanus* populations was confirmed. The results suggest that a *D. africanus* resistant cultivar developed from PC254K1 should be resistant throughout the five localities tested during this study.

**Literature Cited**


Swamy, P. M., and Narasimhareddy, S. 1977. Changes in the leakage of electrolytes from groundnut seeds (*Arachis*


