Neth. J. Pl. Path. 97 (1991) 115-124

# Growth of the mycoparasitic fungus Verticillium biguttatum from different geographical origins at near-minimum temperatures

P.H.J.F. VAN DEN BOOGERT and T.A.W.M. SAAT*

Institute for Soil Fertility Research, P.O. Box 30003, 9750 RA Haren (Gr.), the Netherlands

Accepted: 26 February 1991

**Abstract**

Sclerotia of *Rhizoctonia solani* collected from potato tubers from different countries were assayed for the presence of mycoparasites. Among the mycoparasites observed *Verticillium biguttatum* predominated. Its geographical distribution was not restricted to certain latitudes or soil types; *V. biguttatum* occurred worldwide in potato fields.

The minimum growth temperature of 57 *V. biguttatum* isolates was found to be in the narrow range from 10 to 13 °C, irrespective of their geographical origin. A non-linear logistic growth model was used to describe the radial growth on *Rhizoctonia* mycelium and nutrient agar plates. At near-minimum temperature the maximum colony radii varied considerably; they were up to 3.8 times that of the reference isolate M73. Based on parameter values for logistic growth, fast- and slow-growing isolates could be distinguished. Although the growth properties of *V. biguttatum* isolates from different locations varied, the presence of fast- and slow-growing isolates was not restricted to particular areas and both types could be found in the same field. However, bioassays with selected fast- and slow-growing isolates do not support the assumption that growth at near-minimum temperatures is a relevant criterion for screening isolates of *V. biguttatum* in terms of effectiveness for biological control of *R. solani*.

*Additional keywords*: biological control, logistic growth, *Rhizoctonia solani*.

## Introduction

The mycoparasitic fungus *Verticillium biguttatum* is a potential biological control agent against Rhizoctonia solani in potatoes (Jager and Velvis, 1985, 1986, 1988). Differences in growth response between *R. solani* and *V. biguttatum* hinder successful biological control in the field, as the mycoparasite needs higher temperatures for growth and proliferates more slowly than its host fungus (Van den Boogert and Jager, 1984). To improve its applicability as a biocontrol agent we searched for *V. biguttatum* with a temperature-growth response more similar to that of *R. solani* than isolate M73; the isolate extensively applied in control experiments.

In this paper we report on the results of assays for the presence of *V. biguttatum* on sclerotia from around the world and the analysis of the temperature-growth response of a number of these isolates at near-minimum temperatures.

* Present address: D.J. van der Have B.V., P.O. Box 1, 4410 AA Rilland-Bath, the Netherlands

## Materials and methods

*Acquisition of samples.* Scientists from 15 countries around the world were asked to collect about 100 sclerotia of *R. solani* from locally grown potato tubers, and forward them together with information on soil type, pH and crop rotation. In addition to the imported sclerotia we collected tuber-borne sclerotia from Dutch experimental fields in commom 1:3 crop rotations with potatoes, located at Oudeschip, Haren, Hooghalen, Marknesse and Rolde.

After arrival at our institute, the sclerotia were processed immediately or they were stored at 4 °C for at most three days before use.

*Media and preparation of inoculum.* The following growth media were used: water agar (WA) containing 12.0 g agar (Oxoid No. 3, L 13) per litre tap water; malt peptone agar (MPA) containing per litre deionized water 15.0 g malt extract (Oxoid L 39), 1.0 g peptone (Oxoid L 72) and 12.0 g agar (Oxoid No. 3, L 13). To suppress bacterial growth MPA was supplemented with the filter-sterilized antibiotics oxytetracycline (Vendarcin, Mycopharm, Delft, the Netherlands), streptomycin sulphate (Serva, Heidelberg, FRG) and neomycin sulphate (BDH Chemicals, Poole, UK), each at 50 $\mu$g $ml^{-1}$ medium.

*Rhizoctonia* plates (RP) were prepared as described by Van den Boogert and Gams (1988). Perlite plates, used for incubation of the sclerotia, consisted of water-saturated perlite in 15-cm diam Petri dishes.

Inoculum of *V. biguttatum* for the growth experiments was prepared by punching 6-mm diam mycelial agar disks from the edge of a growing culture on MPA. The conidial suspensions of *V. biguttatum*, used as inoculum source for sclerotia, were made according to Van den Boogert and Gams (1988).

For the bioassays to study the mycoparasitic effectiveness, sclerotia of *R. solani* AG3, isolate 41 AHa, were grown on MPA at 21 °C. After three weeks incubation the developing sclerotial crusts were picked up from the agar layer, followed by rinsing in tap water and air-drying. Three-mm diam disks were punched from the sclerotial crusts to obtain equally sized sclerotia.

*Isolation of V. biguttatum.* The provided sclerotia were carefully transferred to perlite plates and appropriate precautionary measures were taken to prevent undesired contamination. The plates were wrapped in plastic foil and kept at 21 °C in darkness. The sclerotia were examined weekly for the presence of mycoparasites using a dissecting microscope (magnification 32×). As soon as microcolonies of *Verticillium*-like fungi were observed on the sclerotia, subcultures were made on MPA supplemented with antibiotics. After one week of incubation at 21 °C, the isolates were transferred to culture tubes containing antibiotic-free MPA or RP for storage at 4 °C until use.

*Verticillium*-like fungi could easily be recognized in pure culture by their characteristic mat of erect conidiophores. The cylindrical shape of the conidia, their biguttate contents and the absence of chlamydospores served to identify *V. biguttatum*.

Isolate M73 (CBS 228.80) was used as a reference isolate of an effective mycoparasite against *R. solani* (Jager and Velvis, 1985; 1986; 1988).

*Modelling of growth.* Inoculum disks of single isolates were transferred to triplicate

plates and positioned upside-down on the intersection of two perpendicular axes marked on the bottom of the 90-mm diam Petri dishes. At timed intervals the colony radius was measured along the two axes from the intersection point. The net radial extension was the average value of four measurements minus the diameter of the inoculum disk. The following logistic growth model (Pielou, 1969) was used to fit the net radial extensions:

$$R_t = \frac{c}{1 + e^{-b(t-m)}}$$

in which $R_t$ = net radial extension at a given time (mm), $c$ = assymptote, which denotes the maximum colony radius (mm), $b$ = maximum growth rate at $t = m$ (day$^{-1}$), $m$ = acceleration phase which indicates the time required for maximum growth (days)

This approach allows us to characterize the growth kinetics of each isolate by two parameter values ($b$ and $m$). The $c$ parameter value denotes the size of the colony radius after radial growth accomplished on RP or MPA.

The logistic growth parameter values were subjected to univariate (ANOVA) and multivariate (MANOVA) analysis of variance using the Genstat Programs (Rothamsted Experimental Station, Harpenden, UK; release 5). In case the ANOVA was significant, LSD distances were used to trace significant differences between univariate means. If the MANOVA was significant (approximate F statistic), the Mahalanobis distances (canonical variate analysis; approximate t statistics) were used to analyze significant differences between multivariate means.

*Bioassay of mycoparasitic effectiveness.* Selected isolates of *V. biguttatum* with different temperature-growth responses were assayed on cultured sclerotia of *R. solani*. The sclerotia were dipped into a conidial suspension ($0.5 \times 10^6$ conidia ml$^{-1}$) and then transferred to perlite plates for incubation. Suspension liquid without conidia served as a control treatment. Following incubation on perlite for 10 days at 13 °C, the viability of each sclerotium ($N = 50$) was checked by estimating its number of outgrowing hyphae on WA after 24 h incubation at 21 °C. Subsequently, a viability index was calculated according to Velvis et al. (1989).

## Results

*Occurrence of V. biguttatum on sclerotia of R. solani.* The sclerotia from all sources produced hyphae of *R. solani* within 24 h after introduction on moist perlite, indicating that transportation had not markedly affected their viability.

*Verticillium*-like spp. were the most frequently occurring fungi on the sclerotia: they were observed at different frequences in 24 out of 43 samples originating from 11 out of 15 countries (Table 1). Apart from the Peruvian and Argentinian isolates they were all identified as *V. biguttatum*. The *Verticillium*-like species from Peru and Argentina could not be identified as any known species of the genus *Verticillium*: they were added to the Collection of the Centraalbureau voor Schimmelcultures (CBS) as CBS 874.85 and CBS 183.85, respectively. Most Dutch samples listed in Table 2 contained *V. biguttatum*, except the samples from the locations Eemshaven and Hooghalen; these loca-

Table 1. Occurrence of *V. biguttatum* on sclerotia of *R. solani* of different geographic origin.

| Country | Town or region | Collected by | Soil characteristics | | | Previous crop | Sclerotia of *R. solani* | |
|---|---|---|---|---|---|---|---|---|
| | | | type | pH-$H_2O$ | organic matter (%) | | total | frequency with *V. biguttatum* (%) |
| Argentina | San Alberto | A. Ridao | sandy clay loam | 5.6 | 6.4-5.3 | fallow | 50 | 2.0[1] |
| Australia NSW | Conargo | E. Cother | sand | 5.4 | 1.0 | fallow | 76 | 0.0 |
| NSW | Coleambally | E. Cother | sand | 5.0 | 1.0 | potato | 48 | 0.0 |
| NSW | Laggan | E. Cother | dark soil | 6.5 | 10.0 | clover | 86 | 0.0 |
| NSW | Berrigan | E. Cother | sand | 4.9 | 1.0 | potato | 58 | 0.0 |
| NSW | Yanco | E. Cother | sand | 5.8 | 5.0 | potato | 61 | 0.0 |
| NSW | Mangrove Mountain | E. Cother | sand | 6.0 | 2.0 | lettuce | 35 | 0.0 |
| WA | Albany | K. Sivasithamparam | loamy sand | 5.7 | 15-20 | potato | 100 | 0.0 |
| Brazil | Pelotas | C. Castro | clay | 5.0 | 2.0 | strawberry | 100 | 0.0 |
| Canada | Harrington | M. Campbell | loamy sand | 5.8 | 3.5 | cereals | 50 | 4.0 |
| Columbia | Tibaitata 1 | C. de Moreno | – | 5.8 | 6.0 | – | 100 | 3.0 |
| | Tibaitata 2 | C. de Moreno | – | 4.8 | 8.0 | – | 100 | 2.0 |
| | Tibaitata 3 | C. de Moreno | – | 5.9 | 6.4 | wheat | 84 | 27.1 |
| | Tibaitata 4 | L. Nieto Paez | sandy loam | 5.5 | 6.5 | maize | 118 | 11.0 |
| | San Jorge 1 | C. de Moreno | – | 5.3 | 15.7 | – | 100 | 1.0 |
| | San Jorge 2 | C. de Moreno | – | 5.3 | 15.7 | – | 100 | 1.0 |
| | San Jorge 3 | C. de Moreno | – | 5.3 | 15.7 | – | 100 | 0.0 |
| | San Jorge 4 | C. de Moreno | – | 5.3 | 15.7 | – | 100 | 0.0 |
| | Toralapa | G. Claure V. | sandy clay loam | 5.8 | 1.4 | fallow/Lupinus | 100 | 0.0 |
| Denmark | St. Vildmose | J. Hendriksen | peat | – | almost 100 | barley | 39 | 8.6 |
| | Tylstrup | J. Hendriksen | sand | 6.4 | 2.3 | barley | 61 | 41.0 |
| Germany | Gross Lüsewitz 1 | H. Kleinhempel | loamy sand | 5.9 | – | red clover | 47 | 0.0 |
| | Gross Lüsewitz 2 | H. Kleinhempel | loamy sand | 5.7 | – | winter wheat | 58 | 0.0 |
| | Gross Lüsewitz 3 | H. Kleinhempel | loamy sand | 6.7 | – | winter wheat | 48 | 0.0 |
| | Gross Lüsewitz 4 | H. Kleinhempel | loess | 7.3 | 1.6 | winter wheat | 45 | 4.2 |
| Great Britain | Edinburgh | S. Carnegie | clay loam | 6.4 | – | barley | 90 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Netherlands | Creil | P. Bleek | loamy sand | 7.5[2] | 2.0 | gladiolus | 51 | 47.1 |
| | Oudeschip | P. v. d. Boogert | sandy loam | 7.2[2] | – | wheat | 50 | 0.0 |
| | Haren | P. v. d. Boogert | loamy sand | 4.8[2] | 4.6 | wheat | 100 | 78.0 |
| | Hooghalen | P. v. d. Boogert | sand | 4.0[2] | 12.1 | wheat | 50 | 0.0 |
| | Marknesse | P. v. d. Boogert | silt loam | 7.4[2] | 6.9 | wheat | 100 | 37.0 |
| | Rolde | P. v. d. Boogert | sand | 4.9[2] | 4.5 | wheat | 100 | 64.0 |
| | Wieringen | N. v. d. Eijnck | loam | 7.5[2] | 2.6 | barley | 88 | 36.4 |
| | Wijnandsrade | J. Bemelmans | loess | 6.9[2] | 2.0 | barley | 75 | 40.0 |
| Norway | Gjovik | J. Aune | – | 6.3 | – | barley | 50 | 2.0 |
| | Kajajordet | L. Sundheim | clay | 6.4 | 10.0 | cabbage | 100 | 3.0 |
| | Skien | L. Sundheim | – | – | – | barley | 100 | 24.0 |
| Peru | Huancayo | – | – | – | – | – | 50 | 12.0[1] |
| | Huanuco | – | – | – | – | – | 50 | 0.0 |
| Romania | Brasov 1 | B. Plamadeala | sandy clay loam | 6.6 | 12.8 | barley | 100 | 0.0 |
| | Brasov 2 | B. Plamadeala | sandy clay loam | 6.7 | 6.7 | maize | 100 | 4.0 |
| | Brasov 3 | B. Plamadeala | sandy clay loam | 6.5 | 6.7 | maize | 100 | 2.0 |
| | Brasov 4 | B. Plamadeala | sandy clay loam | 6.6 | 6.9 | maize | 100 | 10.0 |
| Sweden | Lund | P. v. d. Boogert | loam | – | – | – | 50 | 24.0 |
| Spain | Alava | A. Monge | – | 7.9 | 3.3 | wheat | 100 | 0.0 |
| USA Alaska | Palmer | D. Carling | silt loam | 6.2 | 8.0-10.0 | barley | 100 | 3.0 |
| Idaho | Ashton | J. Ojala | sandy loam | 7.8 | 1.6-2.2 | barley | 83 | 64.8 |
| Maryland | Beltsville | R. Lumsden | loamy sand | 5.8 | 0.6 | barley/potato | 80 | 0.0 |

[1] Other Verticillium spp.
[2] pH-KCl
– Unknown or not supplied

Table 2. Average logistic parameter values $b$ (maximum growth rate), $m$ (acceleration phase) and $c$ (maximum colony diameter) of *V. biguttatum* isolates ($N = 57$) grouped according to their origin as determined on mycelium of *R. solani* (RP) and on malt peptone agar (MPA) at 13 °C.

| Origin of isolates | Number of isolates | Average logistic growth parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | on RP | | | on MPA | | |
| | | $b \times 100$ | $b/m \times 1000$ | $c$ | $b \times 100$ | $b/m \times 1000$ | $c$ |
| Isolate M73 3 series | 1 | 9.7 | 2.6 | 14.0 | 8.5 | 2.5 | 15.7 |
| Netherlands | | | | | | | |
| Haren | 11 | 9.4 | 2.5 | 18.6 | 10.2 | 3.8 | 15.8 |
| Marknesse | 7 | 8.5 | 2.2 | 28.3 | 13.6 | 6.5 | 19.2* |
| Rolde | 10 | 7.9 | 2.1 | 16.8 | 8.5 | 2.4 | 15.5 |
| various locations | 17 | 10.3 | 2.7 | 16.4 | 12.5 | 5.7 | 15.8 |
| Abroad | | | | | | | |
| San Jorge | 4 | ns | ns | ns | 13.8 | 7.2 | 17.4* |
| various countries | 7 | 9.9 | 2.9 | 17.9 | 12.3 | 5.7 | 12.4 |
| Average | 57 | 9.3 | 2.4 | 18.5 | 11.4 | 4.8 | 16.3 |
| $LSD_{5\%}$ | | 3.4 | 1.1 | 7.0 | 3.4 | 3.1 | 5.2 |

ns = data not supplied.
* = $b/m$ and $c$ significantly different (MANOVA, Mahalanobis distances) from isolate M73 at $P < 0.05$.

tions, however, had recently been reclaimed from sea and woodland, respectively, and potatoes were grown there for the first time.

*Gliocladium roseum*, *G. solani*, other *Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp., *Volutella ciliata*, *Aspergillus* sp. and *Chaetomium* spp. were occasionally observed as inhabitants of the sclerotia. In addition to the fungi, various species of actinomycetes were found, especially after prolonged incubation times.

*Temperature-growth response of V. biguttatum.* At 10 °C, none of the 57 isolates tested showed any radial growth up to 55 days after inoculation. At 13 °C, however, they all did grow on MPA; on RP, however, 11 of the 57 isolates did not grow out. These results were confirmed in an experiment at 23 °C. Seven of these so-called RP-negative specimens originated from the Netherlands; the others were isolates from San Jorge (Columbia) (Table 2). When conidial suspensions were used instead of common agar disks, the RP-negative isolates produced thin colonies on RP with strongly restricted radial extension.

The colony radii varied considerably and ranged from 3.03 to 19.35 mm on MPA and from 2.53 to 13.31 mm on RP after an arbitrary chosen incubation time of 33 days.

*Growth characterization.* As the variation between the triplicate net coloni radii of the tested isolates was extremely low (5.0% of the total variance), further calculations were carried out with the average values. The averages of the net coloni radii were fitted to the logistic growth model $R_t$. This approach adequately described the radial growth of the various isolates on agar plates; on average, 99.1% ($N$ = 57; 97.0-100%) of the total variance of each isolate could thus be explained.

The $b$ parameter values from the MPA and RP series were negatively correlated with $m$ (significant at $P < 0.01$; $R = -0.880$ and $R = -0.584$ on MPA and RP, respectively) and weakly correlated (not significant; $R = 0.077$ and $R = -0.097$ on MPA and RP, respectively) with $c$. Therefore, the ratios $b/m$ and $c$ of each isolate were considered

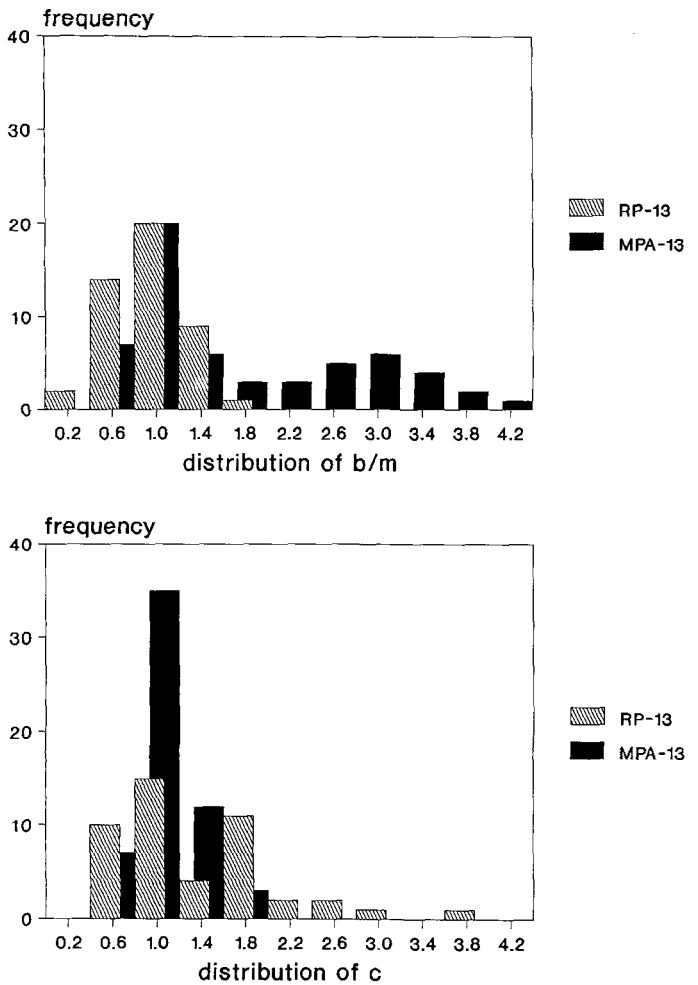


Fig. 1. Frequency distribution (class size 1.0 ± 0.2) of the growth properties of different isolates of *V. biguttatum* as determined on mycelium of *R. solani* (RP) and malt peptone agar (MPA) at 13 °C; growth kinetics ($b/m$; top) and colony radii ($c$; bottom) relative to reference isolate M73.

Table 3. Effect of various isolates of *V. biguttatum* on the viability of sclerotia of *R. solani* ($N$ = 50) after 10 days of incubation at 13 °C.

| Isolates of *V. biguttatum* | Viability index (0-100) |
|---|---|
| Control (no *V. biguttatum*) | 99.3 |
| M73 | 0.7 |
| Fast-growing isolates | |
| INT-3 (Norway) | 8.3 |
| INT-20 (Germany) | 1.5 |
| INT-25 (Denmark) | 9.5 |
| Slow-growing isolates | |
| HAREN 16 (Netherlands) | 3.9 |
| ROLDE 17 (Netherlands) | 2.3 |

as characteristics for their growth kinetics and maximum colony radii, respectively.

The frequency distributions of *b/m* and *c* (relative to reference isolate M73) are presented in Figure 1. The highest frequency coincided with the class interval of M73 (1.0 ± 0.2) and approximately 40% of the isolates showed deviating growth properties up to 4.2 times the growth kinetics and 3.8 times the maximum growth radius of isolate M73.

*Geographical distribution in relation to growth properties.* The isolates were grouped according to their geographical origin (Table 2). The MANOVA of *b/m* and *c* indicated that the average growth properties (*b/m* and *c*) on RP and MPA ($N$ = 57) were not significantly different whereas there were considerable differences between the groups on MPA: the Marknesse and San Jorge isolates were relatively fast-growing compared with M73 (approximate $t$ statistics, significant at $P$ = 0.05). Based on arbitrary chosen limits of the growth kinetics, fast-growing (*b/m* >1.4 × *b/m* of M73) and slow-growing (*b/m* <0.6 × b/m of M73) isolates could be distinguished.

Representatives of both fast- and slow-growing isolates were obtained from the sites of Haren, Marknesse and Rolde; the San Jorge isolates were all fast-growing.

*Mycoparasitic effectiveness of fast- and slow-growing isolates.* The growth kinetics (*b/m*) on MPA an RP did not show similar values; in fact, no correlation existed between the paired *b/m* values of each isolate ($R$ = −0.01). Some extremely fast- and slow-growing specimens (as previously described) were selected from the isolates that would grow on RP to assess their mycoparasitic effectiveness. The bioassay showed that slow-growing isolates were not less effective against *R. solani* sclerotia than the fast-growing isolates at near-minimum temperature (Table 3).

## Discussion

*Verticillium biguttatum* was by far the most frequently occurring mycoparasite found on sclerotia of *R. solani* from potato tubers. The geographical distribution of *V. biguttatum* appears to be worldwide. This is substantiated by the fact that Dr K. Sivasitham-

param and Dr J. Coley-Smith found *V. biguttatum* also in Australia and the United Kingdom, respectively (personal communication). The results also show that the occurrence of *V. biguttatum* is not restricted to a particular soil type or soil pH. Apparently the fungus is able to survive in different soil types, varying from almost purely mineral soils to peat soils, at pH values between 4.8 and 7.9.

The frequencies of *Verticillium*-positive samples listed in Table 1 (51.1%, $N = 43$) differ markedly from those observed in the northern part of the Netherlands (98%, $N = 52$; Jager and Velvis, 1980). Shipping conditions (air-drying, temperature fluctuations) may have affected *V. biguttatum* negatively, although the supporting sclerotia could withstand such conditions. The difference in frequency between the foreign and Dutch samples may also be caused by the high cropping frequency of potatoes in combination with the common use of nematicides. Such management practices, which are presumably more intensive in the Netherlands than in many other countries, favour the development of *R. solani* and consequently *V. biguttatum* (Hofman, 1988).

Radial growth on agar plates could satisfactorily be described by the logistic growth model $R_t$, which enabled us to evaluate growth rate separate from the maximum colony diameter of each isolate. In this approach all data points were used instead of the usual, arbitrary chosen linear part of the growth curve (Van den Boogert and Jager, 1984).

The minimum temperature for growth of *V. biguttatum* was found to be in the narrow range between 10 and 13 °C. Large variations in growth properties were shown in natural populations of *V. biguttatum*. Based on the results of the bioassays with fast- and slow-growing isolates, it is doubtful, however, whether differences in growth rate at near-minimum temperature are of practical significance in as far as biological control of *R. solani* in the field (Jager and Velvis, 1985; 1986) or in storage (Jager and Velvis, 1988) is concerned.

Although *V. biguttatum* was isolated from tuber-borne sclerotia of *R. solani*, part of the isolates showed weak radial extension on mycelium of *R. solani*. Possibly, differences may exist in susceptibility of *R. solani* to *V. biguttatum*. The large variation in the logistic parameter for maximum colony diameter may be interpreted in terms of variation in susceptibility of *R. solani* to mycoparasitism. Differences in host susceptibility have also been reported for the mycoparasitic *Trichoderma* species (Naiki, 1986; Köhl, 1989).

We found isolates of *V. biguttatum* with different growth properties. The similar mycoparasitic capacities of fast- and slow-growing isolates, however, suggest that growth properties at near-minumum temperature are not the best criteria for selection of effective isolates.

**Acknowledgements**

This investigation was supported by the National Foundation for Technical Research (STW), the technical division of the Dutch Organization for Scientific Research (NWO). We thank Dr P.C. de Ruiter for his suggestions on data analysis. The manuscript was critically reviewed by Dr W. Gams.

## References


Boogert, P.H.J.F. van den & Gams, W., 1988. Assessment of *Verticillium biguttatum* in agricultural soils. Soil Biology and Biochemistry 20: 899-905.

Boogert, P.H.J.F. van den & Jager, G., 1984. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 3. Inoculation of seed potatoes with different fungi. Netherlands Journal of Plant Pathology 90: 117-126.

Hofman, T., 1988. Effects of granular nematicides on the infection of potatoes by *Rhizoctonia solani*. Thesis Wageningen Agricultural University, Wageningen, the Netherlands.

Jager, G. & Velvis, H., 1980. Onderzoek naar het voorkomen van *Rhizoctonia*-werende aardappelpercelen in Noord-Nederland. Inst. Bodemvruchtbaarheid, Rapp. 1-80, 60 pp. (With an English summary: Occurrence of *Rhizoctonia*-suppressing potato fields in the northern part of the Netherlands).

Jager, G. & Velvis, H., 1985. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 4. Inoculation of seed tubers with *Verticillium biguttatum* and other antagonists in field experiments. Netherlands Journal of Plant Pathology 91: 49-63.

Jager, G. & Velvis, H., 1986. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 5. The effectiveness of three isolates of *Verticillium biguttatum* as inoculum for seed tubers and of a soil treatment with a low dosage of pencycuron. Netherlands Journal of Plant Pathology 92: 231-238.

Jager, G. & Velvis, H., 1988. Inactivation of sclerotia of *Rhizoctonia solani* on potato tubers by *Verticillium biguttatum*, a soil-borne mycoparasite. Netherlands Journal of Plant Pathology 94: 225-231.

Köhl, J., 1989. Eignung von Stämmen aus der Gattung *Trichoderma* für die biologische Bekämpfung phytopathogener Pilze. Thesis. Justus-Liebig University, Giessen, Germany.

Naiki, T., 1986. Differences in susceptibility of sclerotia of *Rhizoctonia solani* Kühn to *Trichoderma* sp. Soil Biology and Biochemistry 18 : 551-553.

Pielou, E.C., 1969. An introduction to mathematical ecology. Wiley-Interscience, New York etc., 286 pp.

Velvis, H., Boogert van den, P.H.J.F. & Jager, G., 1989. Role of antagonism in the decline of *Rhizoctonia solani* inoculum in soil. Soil Biology and Biochemistry 21: 125-129.