

Full Length Research Paper

Effect of endophytic *Fusarium oxysporum* on paralysis and mortality of *Pratylenchus goodeyi*

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Three bioassays were conducted to investigate the antagonistic effect of secondary metabolites produced by 5 endophytic *Fusarium oxysporum* isolates from banana (*Musa* spp.) plants in Kenya, against *Pratylenchus goodeyi*. Percentage paralysees were recorded 3, 6 and 24 h after exposure to culture filtrates. Percentage mortality was evaluated after 48 h. All isolates caused significantly higher percentage paralysis (17.5 - 25.9%) and percentage mortality (62.3 - 72.8%) of *P. goodeyi* motile stages compared to the control (8.4 - 10.9% and 17.3 - 34.6%, respectively). Percentage paralysis of motile stages of *P. goodeyi* decreased as the length of time exposure to culture filtrates increased, while mortality increased as length of nematodes exposure to culture filtrates increased. Kenyan isolates performed equally as good as the Ugandan isolate (V5W2) in causing paralysis and mortality. Results from this study demonstrated that endophytic *F. oxysporum* antagonizes *P. goodeyi* through production of secondary metabolites.

Key words: Banana, endophyte, *Fusarium oxysporum*, mortality, paralysis.

INTRODUCTION

Banana (*Musa* spp.) is an important staple food for millions of people in developing countries of the tropics (INIBAP, 1991). In Kenya, it is an important food and cash crop for millions of rural farmers in banana producing areas, grown on an estimated 2% of total arable land predominantly by small-scale farmers (Seshu et al., 1998). Production is mainly characterised by low input application, with no or limited use of pesticides and fertilizers (Swennen and Vulysteke, 2001). Consequently, banana production in Kenya has been on a very rapid decline over the last 2 decades (MOALD, 1994). This sharp decline in yield has been due to the combined effect of pests and diseases, including nematodes, which have reduced average Kenyan banana yields on traditional farms to 14 ton/ha, less than one-third of the crop's potential under humid tropical conditions (Karamura, 1998). Bananas in East Africa are attacked by a complex of nematodes which include burrowing nematodes (*Radopholus similis* (Cobb) Thorne), the root lesion

nematode (*Pratylenchus goodeyi* (Sher and Allen)) and the spiral nematode (*Helicotylenchus multicinctus* (Cobb) Golden) (Gowen and Quénehervé, 1990a; Karamura, 1993). The lesion nematode *P. goodeyi* (Sher and Allen) has been observed in many banana growing region in Kenya and can cause yield losses of over 85% (Bridge et al., 1997; Gichure and Ondieki, 1977; Gowen and Quénehervé, 1990a; Gowen and Quénehervé, 1990b). Damages caused by *P. goodeyi* lead to stunting, lengthened vegetative cycles, a reduction in number of leaves and bunch weight and eventual toppling over, especially in soils with low nutritional content (Bridge et al., 1997). The use of contaminated planting material to establish new fields is the main source of *P. goodeyi* infestation in banana fields (Sarah, 1989). Due to high cost of nematicides combined with their related health and environmental concerns, there is need for alternative methods of plant-parasitic nematode management.

The potential of endophytic *Fusarium oxysporum* isolates, naturally occurring inside banana plants, has gained attention as an alternative to nematicides (Athman et al., 2006; Athman, 2006; Dubois et al., 2006; Dubois et al., 2004; Sikora et al., 2003). Endophytes are microorganisms that spend part or all of their life cycle residing

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benignly inside host plant tissues (Wilson, 1995). Many endophytes form mutualistic relationships with their host plants, from which they obtain nutrients and in turn confer protection against biotic and abiotic stresses to the plant (Schulz and Boyle, 2005). When inoculated into otherwise sterile banana tissue-cultured plants, populations of and damage caused by *Radopholus similis* (Cobb) Thorne is reduced (Athman, 2006; Dubois et al., 2004; Paparu et al., 2004; Paparu et al., 2006). Endophytic *F. oxysporum* protects banana plants by induction of systemic resistance (Paparú et al., 2006) and production of secondary metabolites with nematicidal properties, causing *R. similis* mortality and paralysis (Charbonneau, 1997; Dubois et al., 2004). Among several Ugandan isolates tested, V5w2 caused the highest percentage *R. similis* paralysis (100%) and mortality (100%) (Athman, 2006) and is currently being investigated in on-farm experiments (REF). Although extensive research has been carried out on the use of endophytic *F. oxysporum* in management of *R. similis* in bananas, no study has investigated the potential of endophytic fungi against *P. goodeyi*. The objective of this study was to 1) investigate the effect of secondary metabolites produced by 5 endophytic *F. oxysporum* isolates from banana plants in Kenya, on mortality and paralysis of *P. goodeyi* and 2) compare it to that of the Ugandan strain V5w2, which is antagonistic to *R. similis*.

MATERIALS AND METHODS

Endophytes

Five endophytic *F. oxysporum* isolates (4M0C321, 2MR23, 5JTOC134 and 5MR11) originating from banana plants in Meru, Kenya (Mwaura et al., 2008) and one endophytic *F. oxysporum* isolate (V5w2), originating from banana plants in xx, Uganda (REF) were included in the study. The isolates had been preserved on filter paper in sterile 2.5 ml Eppendorf tubes at 4°C. For each isolate and under sterile conditions, a filter paper was placed on sterilized (autoclaved at 121°C for 15 min) synthetic nutrient agar medium (SNA) (1 g KH₂PO₄, 1 g KNO₃, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 0.6 ml NaOH (1 M), 13.2 g agar/l sterile distilled water (SDW)) in 90 mm diameter petri dishes. Petri dishes were incubated in the laboratory (-25°C with a natural photoperiod of -12:12 L: D h) for 7 days. For each isolate, 3 blocks of SNA (1 cm³) containing spores and mycelium were aseptically inoculated into 250 ml sterile Erlenmeyer flasks containing 100 ml of sterilized half strength potato dextrose broth medium (PDB) (12 g PDB/l SDW; Sigma-Aldrich, City, Country) and flasks containing inoculum were incubated for 7 days in the laboratory. Filtrates from each isolate were obtained by filtering PDB through cheese cloth into sterile 40 ml plastic bottles and centrifuged at 2,012 g for 15 min. One ml of each filtrate was transferred aseptically into 7 ml Bjorn glass bottles, after which pH was recorded.

Nematodes

Nematode-infested roots were obtained from Cavendish banana fields (Juja, Kenya), transported in a cool box to the laboratory. Roots were washed under running tap water to remove soil, cut into

1 cm long pieces and macerated in using a Kenwood blender (Kenwood, UK) for 15 s. The macerated suspension was collected in a modified Baermann dish and nematodes extracted overnight (Luc et al., 1990). Nematodes that collected at the bottom of the dish were transferred into a 10 ml beaker, concentrated using a 28 µm (aperture) sieve and counted per sex (approximately the composition was, 21 female, 16 male and 12 juveniles) male female juvenile ratio and mention adjusted to 50 mixed stages/ml by either concentrating the suspension or diluting the suspension containing the nematodes.

Bioassays

A total of 3 repeated bioassays were conducted. Bioassays consisted of 6 treatments: 4 treatments comprised the fungal isolates, while 2 treatments were controls (SDW and PDB with pH adjusted to the average pH from all the fungal culture filtrates). Each bioassay was arranged in a complete randomized design and conducted in the laboratory. Within each bioassay, each treatment was repeated 3 times, using 3 bottles. Culture filtrates were inoculated with 50 mixed stages (female, male and juveniles) of *P. goodeyi* suspended in 100 µl SDW.

Mortality and paralysis was determined after 3, 6 and 24 h of exposure to culture filtrates. Active nematodes were considered those with normal sigmoid shape and exhibiting active movement; paralyzed nematodes were considered those that were not active and that were curved and never in a straight position even after poking them with a fine sterile needle, while dead nematodes were considered those that were straight (uncurved) after poking them with fine needle, the percentage dead and paralyzed nematodes were calculated based on the original numbers of nematodes.

After 24 h exposure to culture filtrates, nematodes were concentrated through a 28 µm sieve, rinsed with SDW and transferred into sterile 7 ml Bjorn bottles containing 2 ml SDW. The nematode cultures were left in the laboratory for an additional 24 h. Nematodes were probed with a fine needle under a compound microscope (magnification × 20) and those that were elongated and remained immotile even after probing with a fine needle were considered dead. Percentage mortality and paralysis were calculated using Abbotts corrected mortality formula (Abbott, 1925).

Statistical analysis

Using percentages from the water control treatment, percentages mortality and paralysis from each of the other treatments were corrected using Abbott's formula (Abbott, 1925). Corrected percentages mortality and paralysis were arcsine-square root transformed before analysis of variance (ANOVA) to obtain equal variances among treatments and normal distributions. Two way ANOVA was used to demonstrate interaction effects between treatments and exposure time. When interactions were significant, the effects of one factor were analysed at each level of the interacting factor. If significantly different, treatment means were compared using Tukey test (SAS, 2001).

RESULTS

Effect of isolates culture filtrates on *P. goodeyi* paralysis

Percentage paralysis (immobilization) caused by culture filtrates of different *F. oxysporum* isolates differed among the 3 bioassays ($P < 0.001$) with interaction between

Table 1. Percentage paralysis (immobilization) (mean \pm S.E.) of *P. goodeyi* mixed stages (males, females and juveniles) in culture filtrates of 6 endophytic *F. oxysporum* isolates after exposure times of 3, 6 and 24 h.

Isolate	Bioassay 1			Bioassay 2			Bioassay 3		
	3 h	6 h	24 h	3 h	6 h	24 h	3 h	6 h	24 h
5JTOC134	34.8 \pm 3.4 ^a	31.0 \pm 0.8 ^a	19.9 \pm 1.1 ^{ab}	34.9 \pm 3.4 ^a	31.0 \pm 0.8 ^a	19.9 \pm 1.0 ^{ab}	27.6 \pm 5.5 ^b	10.8 \pm 5.9 ^{cd}	6.30 \pm 3.5 ^a
4M0C321	28.2 \pm 2.9 ^{ab}	24.5 \pm 1.4 ^a	23.3 \pm 2.6 ^a	28.6 \pm 2.9 ^a	26.2 \pm 2.1 ^{ab}	24.4 \pm 3.3 ^a	28.2 \pm 0.3 ^b	21.7 \pm 1.2 ^{abc}	16.7 \pm 2.0 ^a
5MR11	27.8 \pm 4.6 ^{ab}	27.2 \pm 5.7 ^a	17.6 \pm 3.3 ^{ab}	27.9 \pm 4.5 ^{ab}	27.2 \pm 5.7 ^a	17.6 \pm 3.2 ^{ab}	41.9 \pm 1.7 ^a	30.9 \pm 1.3 ^a	15.5 \pm 3.3 ^a
V5W2	16.2 \pm 8.7 ^{ab}	16.1 \pm 1.0 ^a	6.4 \pm 6.4 ^b	26.4 \pm 4.6 ^{ab}	24.8 \pm 2.5 ^{ab}	16.4 \pm 2.8 ^{ab}	29.9 \pm 2.1 ^{ab}	24.9 \pm 0.8 ^{ab}	20.3 \pm 0.4 ^a
2MR23	11.8 \pm 2.5 ^b	17.8 \pm 0.9 ^a	20.7 \pm 1.5 ^{ab}	11.9 \pm 2.4 ^{bc}	18.0 \pm 0.8 ^{abc}	20.9 \pm 1.7 ^{ab}	28.1 \pm 2.8 ^b	16.5 \pm 1.6 ^{bcd}	10.8 \pm 5.4 ^a
PDB	12.4 \pm 2.7 ^b	12.9 \pm 3.1 ^a	12.7 \pm 1.7 ^{ab}	12.4 \pm 2.7 ^{bc}	12.9 \pm 3.1 ^{bc}	12.7 \pm 1.7 ^b	9.9 \pm 1.5 ^c	12.9 \pm 1.4 ^{bcd}	14.0 \pm 2.1 ^a
Water	10.3 \pm 1.7 ^b	10.4 \pm 1.8 ^a	13.4 \pm 0.8 ^{ab}	10.3 \pm 1.7 ^c	10.4 \pm 1.8 ^c	13.3 \pm 0.8 ^{ab}	4.5 \pm 2.3 ^c	6.7 \pm 3.8 ^d	6.10 \pm 3.3 ^a

treatments and bioassays ($F = 3.5$, $DF = 12$, $P = 0.001$). In individual bioassays, percentage paralysis of *P. goodeyi* was influenced by the 3 different time of exposure ($F = 15.0$, $DF = 2$, $P < 0.001$). As the length of exposure time increased, percentage paralysis decreased.

In all the 3 bioassays conducted, percentage *P. goodeyi* nematodes immobilized by some *Fusarium* culture filtrates were significantly higher than the control treatments (Table 1). In bioassay 1, after 3 h exposure, percentage paralysis by fungal filtrates did not differ from each other neither did they differ from the control ($P = 0.0054$). However, compared to bioassay 3, after 3 h of exposure, all the fungal isolates except 2MR23 differed from one control (Water) (Table 1). Over all the isolate 5JTOC134 resulted in nematode immobilization rates similar to that of the reference isolate V5W2 (Table 1) across bioassays.

Effect of isolates culture filtrates on *P. goodeyi* mortality

Fusarium isolates caused variable ($P < 0.001$)

mortality levels on motile stages of *P. goodeyi* after 48 h of exposure to culture filtrates of the isolates. Interaction between the treatments and bioassays was significant ($F = 11.9$, $DF = 12$, $P < 0.001$) meaning that the same isolates caused varied mortality levels across the three repeat bioassays. In bioassay 1, the reference isolate V5w2 caused significantly higher mortality (75.9%) compared to the controls (42.1 and 22.2%) PDB and water respectively. The 2 controls had significant differences, with more mortality in PDB control as opposed to water control. Although V5w2 had the highest effect on the mortality of the nematodes, its effects did not differ from the Kenyan isolate 5JTOC134 in causing mortality (Figure 1). In bioassay 2, isolate 2MR23 caused the highest mortality (77.5%) and again, the isolate did not significantly differ from the tester isolate V5w2 (Figure 1). However all the isolates differed significantly from the 2 controls. In bioassay 3, isolate 5JTOC134 had significantly higher mortality (81.4%), but had similar effect to the tester strain V5w2 (Figure 1). Control treatment (Water) had lower nematode mortality compared with the pH adjusted PDB control in

bioassays 1 and 2, while in bioassay 3, the controls did not differ from each other ($P = 0.05$).

DISCUSSION

The study revealed that all the culture filtrates of the 5 endophytic *F. oxysporum* isolated from banana plants in Kenya had *in vitro* mortality and paralysis effects on *P. goodeyi*. Although previous experiments focused more on effects of endophytic *F. oxysporum* against exotic nematodes in Africa, no research has focused on the effect of endophytic fungi isolated from Kenyan bananas against the African native lesion nematode, *P. goodeyi*. It is clear that isolates tested in the study produced nematicidal or nematotoxic compounds against *P. goodeyi*, with results independent upon parameters such as the fungal isolate, pH or the concentration of the culture filtrates.

All efforts were taken to maintain constant conditions for the 3 repeat bioassays, but there were variations that occurred within treatments in different bioassays. There are a number of factors that could have contributed to the inconsistencies,

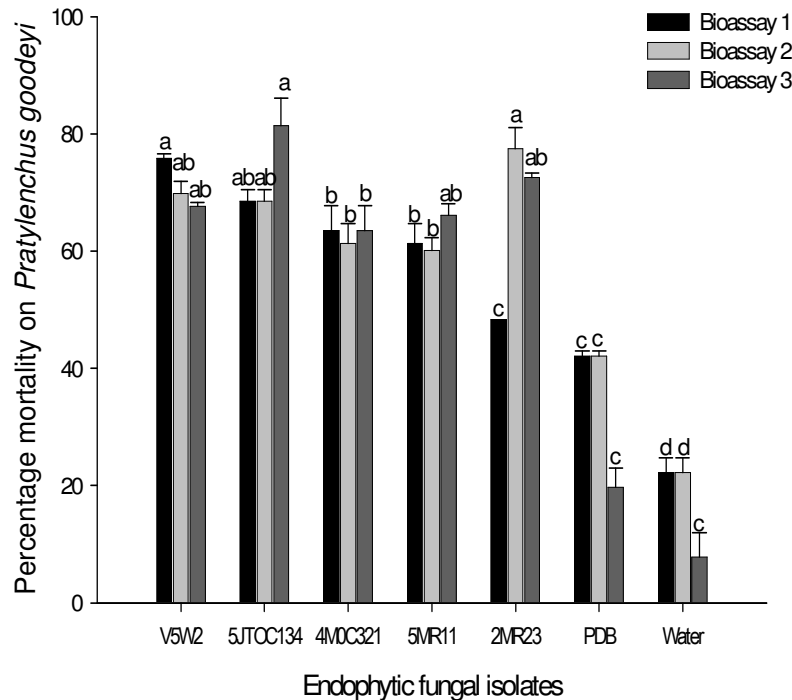


Figure 1. Percentage mortality (mean \pm S.E.) of *P. goodeyi* mixed stages (males, females and juveniles) in culture filtrates of 5 endophytic *F. oxysporum* isolates after an exposure time of 24 h, n = 3, in 3 repeat bioassays.

such as the type of culture medium, age of the fungal culture filtrates, culture filtrate concentration and the time of nematodes exposure to culture filtrates (Cayrol et al., 1989). The pH of the fungal filtrates on nematodes inhibition seems to have an effect since there was differences in mortality between the pH adjusted control and water control. Partly, the effect could be attributed to the to the levels of osmotic pressure created by the dextrose in PDB, which could have lead nematodes desiccation and eventual death due to osmotic pressure difference. However, there is need to evaluate further the effect of using the PDB as a control as compared to other suitable controls.

Isolates 5JTOC134 and 5MR11 resulted in consistency in causing both mortality and paralysis of *P. goodeyi* and were comparable to the tester strain V5W2, which has been reported to be the best isolate against *R. similis* (Athman, 2006; Dubois et al., 2004). This also demonstrates that fungal endophytes e.g. V5W2 produces compounds which can cause mortality and paralysis to multiple hosts (*R. similis* and *P. goodeyi*) as opposed to earlier studies on effects on nematocidal properties of culture filtrate of the nematophagous fungus *Paecilomyces zilacinus*, which was only found to have specific effects on nematodes of Heteroderidae family (*Meloidogyne*, *Heterodera*) (Cayrol et al., 1989).

The time of exposure had a vital role in influencing both the mortality and paralysis of the nematodes. It was evident that the toxic effects of the isolates after 24 h of

exposure to culture filtrates of the isolates were not reversible even after rinsing the nematodes with clean sterile distilled water. However toxic effects of some endophytic *F. oxysporum* have been reported to have a reversibility effect if nematodes were exposed to 6 h or less (Athman, 2006). Percentage paralysis of the nematodes decreased as time of exposure increased. The total number of paralysed nematodes translated into dead nematodes as time length increased. This indicates that nematodes are first paralysed before they eventually die off as a result of toxic nature of the culture filtrates of endophytic fungi. The fact that the isolates caused both mortality and paralysis to all the motile stages of *P. goodeyi* is of great importance since both sexes of the nematodes are invasive to banana plants (Gichure and Ondieki, 1977).

Currently there is very limited information on the nematodes-inhibiting nature of the fungal culture filtrates, especially those antagonistic to the nematodes. However, it was evident that the compounds in the culture filtrates used in this study were toxic to *P. goodeyi* since the filtrates did not contain any spores and mycelia. PDB used as the control in this experiment had negligible effects on the nematodes mortality since it varied significantly with all the isolates at different times of exposure, indicating that the effects were independent of the PDB effect.

The study revealed the potential of endophytic fungi as biological control agent against the lesion nematode, *P.*

goodeyi. The study revealed that all the culture filtrates of the 5 endophytic *F. oxysporum* isolated from banana plants had *in vitro* mortality and paralysis effects on *P. goodeyi*. *F. oxysporum* as control agent of banana nematode *P. goodeyi*.

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REFERENCES

- Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Athman SY, Dubois T, Viljoen A, Labuschagne N, Coyne D, Ragama P, Gold CS, Niere B (2006). *In vitro* antagonism of endophytic *Fusarium oxysporum* isolates against the burrowing nematode *Radopholus similis*. *Nematol.* 8: 627-636.
- Athman YS (2006). Host-endophyte-pest interactions of endophytic *Fusarium oxysporum* antagonistic to *Radopholus similis* in banana (*Musa spp.*). PhD Thesis, University of Pretoria, Pretoria, South Africa.
- Bridge J, Fogain R, Speijer P (1997). The root lesion nematodes of banana. *Musa pest fact sheets*. pp.1-4.
- Cayrol J, Djan C, Pijarowski L (1989). Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue Nematologie* 12: 331-336.
- Charbonneau P (1997). Debunking endophytes [Online] <http://www.gov.on.ca/OMAFRA/english/crops/facts/endophyt.htm>.
- Dubois T, Gold CS, Paparu P, Athman S, JTD Silva, Kapindu S (2006). Tissue culture and the *in vitro* environment. Enhancing plants with endophytes: Potential for ornamentals, Floriculture, ornamental and plant biotechnology: Advances and topical Issues, 1st Edition. Global Science Books, London, UK. pp. 397-409.
- Dubois T, Gold CS, Paparu P, Mukwaba E, Athman S, Kapindu S, Adipala E (2004). Merging biotechnology with biological control: Banana *Musa* tissue culture plants enhanced by endophytic fungi. *Uganda J. Agric. Sci.* 9: 441-451.
- Gichure E, Ondieki JJ (1977). A survey of banana nematodes in Kenya. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz.* 84: 724-728.
- Gowen SR, Luc M, Quénehervé P (1990a). Nematode parasites of banana, plantains and Abàca, *Plant Parasitic Nematodes in Sub Tropical and Tropical Agriculture*. CAB International, Wallingford, UK. pp. 431-460
- Gowen SR, J Bridge, Quénehervé P (1990b). Nematode parasites of bananas, plantains and abaca. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford. pp. 431- 460
- INIBAP (1991). Regional Network for Eastern Africa. INIBAP, Montpellier, France.
- Luc M, Sikora RA, Bridge J (1990). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford. pp. 45-68.
- MOALD (1994). Annual Reports- Kirinyaga, Kisii and Murang'a Districts. Ministry of Agriculture. Government of Kenya, Nairobi.
- Mwaura PM, Kahangi EM, T Losenge, Dubois T, Coyne D (2008). Isolation and screening of Endophytic *Fusarium oxysporum* for biological control of nematodes in tissue cultured bananas.
- Paparu P, Dubois T, Gold CS, Adipala E, Niere B, Coyne D (2004). Inoculation, colonization and distribution of fungal endophytes in *Musa* tissue culture plants. *Uganda J. Agric. Sci.* 9: 583-589.
- Paparu P, Dubois T, Gold CS, Niere B, Adipala E, Coyne D (2006). Colonization pattern of nonpathogenic *Fusarium oxysporum*, a potential biological control agent, in roots and rhizomes of tissue cultured *Musa* plantlets. *Ann. Appl. Biol.* 149(1):1-8.
- Sarah JL (1989). Banana nematodes and their control. *Nematropica* 19: 199-216.
- SAS (2001). The SAS System, Version 8.2, for Windows. SAS Institute Insitute Inc, USA. 1: p. 943.
- Schulz B, Boyle C (2005). The endophytic continuum. *Mycol. Res.* 109: 661-686.
- Sikora RA, Niere B, Neuenschwander P, Kimenju J (2003). Endophytic microbial biodiversity and plant nematode management in Africa agriculture. *Biological Control in IPM systems in Africa*, CAB International, Wallingford. pp. 179-192
- Swennen R, Raemaekers RH, Vulysteke D (2001). Banana, *Crop Production in Tropical Africa*. Directorate General for International Co-operation (DGIC). Brussels, Belgium. pp. 530-552,
- Wilson D (1995). Endophyte-the Evolution of a term, and clarification of its use and definition. *Oikos* 73: 274-276.