Control by arbuscular endomycorrhizae of *Pratylenchus brachyurus* in pineapple microplants

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Pratylenchus brachyurus (Godfrey) Filip & Schurr-Steekh. has been reported in association with pineapple roots and is considered as an important pathogen on pineapple. Microplants of Queen Tahiti, Smooth Cayenne and Spanish varieties were inoculated with *Glomus* sp. (LPA21) and/or *P. brachyurus* at transplanting from axenic conditions or one month later. The presence of the nematode did not affect shoot growth of endomycorrhizal plants. Late *P. brachyurus* inoculation did not influence growth of nonmycorrhizal plants while early pathogen application caused reductions in nonmycorrhizal plant growth. Nematode number per g of root was significantly decreased for endomycorrhizal plants when pathogen was introduced at outplanting or one month later. Nematode inoculation affected endomycorrhizal colonization estimated by non vital staining for the Queen Tahiti and Spanish varieties but did not alter development of metabolically active arbuscules in roots of the three varieties. P concentration of endomycorrhizal shoots was higher for all treatments and *P. brachyurus* tended to decrease mineral concentration of nonmycorrhizal plants with early nematode application.

Key words: *Ananas comosus,* endomycorrhizal infection, *vitro*plant, pathogen nematode, interactions, integrated control

Introduction

Arbuscular endomycorrhizal fungi (AMF) and soilborne pathogens occur together in the rhizosphere around plant roots. Arbuscular endomycorrhizae can positively influence plant development by improving mineral nutrition, water uptake, hormone production, resistance to root pathogen or tolerance to pesticides (GIANINAZZI et al. 1982). GUILLEMIN et al. (1991) have shown the benefits to the growth of pineapple microplants of inoculation with endomycorrhizal fungi.

Nematodes are considered an important factor in reducing pineapple production. *Pratylenchus brachyurus* (Godfrey) Filip & Schurr-Steekh. causes widespread damage in pineapple plantations, particularly in the Ivory Coast. It was described for the first time in pineapple roots by GODFREY (1929) in Hawaii. This nematode penetrates the elongation zone of roots and develops in the cortical tissue and vascular cylinder (GUÉROUT 1975). Secondary roots can then be destroyed by nematode infestation giving a root system that is essentially composed of primary

roots (CASWELL et al. 1990). This endopathogen also modifies vegetative plant growth, with reductions in leaf area, and causes a delay in shoot development (LACOEUILHE and GUÉROUT 1976, KEETCH 1982). Decreases in fruit yield can be in the region of 30 to 35% and the number of suckers may be reduced to 80% (LACOEUILHE and GUÉROUT 1976, KEETCH 1982). *P. brachyurus* has a high impact in the Ivory Coast (GUÉROUT 1975), because pineapple is often grown in soil with a pH adapted to nematode proliferation (pH 5 to 5.5) (SARAH 1991).

The potential of AMF to alleviate nematode-induced plant stress has been previously investigated in different plant species but variable host-plant responses to pathogen-endomycorrhiza interactions have been reported (e.g. BAGYARAJ et al. 1979, KELLAM and SCHENCK 1980, CASON et al. 1983, ELLIOT et al. 1984, COOPER and GRANDISON 1986, SMITH 1987, INGHAM 1988, THOMAS et al. 1989). In the present work, we have tested interactive effects of AMF and *P.brachyurus* on plant growth, endomycorrhizal infection development and root-colonising nematode populations of micropropagated pineapple.

Material and methods

Three micropropagated pineapple varieties (*Ananas comosus* (L.) Merr., Queen Tahiti, Smooth Cayenne (clone CY0) and Spanish varieties) were tested. Plants were raised in a growth chamber under simulated tropical conditions (300µE s^{-1} m^{-2}, 29–25°C, 12h day and 70%–90% relative humidity) in a γ-irradiated (10kGy) acid soil (pH 5.0). Pineapple microplants were inoculated with root fragments of *Tephrosia ehlenbergiana* infected with an isolate of *Glomus* sp. (LPA21) in trays containing a soil:gravel (1:1, v:v) mix during a four-week acclimatization period (M) (GUILLEMIN et al. 1991) or when one month-old weaned microplants were transplanted individually to pots (1+M). Each pot contained 400g of the soil:gravel mix and was watered daily with distilled water and weekly with 2x20 ml of Hoagland n°2 solution (HOAGLAND and ARNON 1950) without phosphate.

Inoculation of *Pratylenchus brachyurus* was performed with about 100 nematodes per microplant at outplanting from axenic conditions (Nematode) or one month later at the end of the weaning period (Nematode+1).

After 3 months in pots, several growth parameters were evaluated: leaf area (cm^2), shoot and root fresh mass (g) and shoot dry mass (g) and the N, P, K, Ca and Mg concentrations of shoots determined (WARNER and JONES 1967, Comité Inter Instituts pour le diagnostic foliaire 1968, 1972). Endomycorrhiza development was estimated microscopically by the method of TROUVELOT et al. (1986) after clearing roots and staining fungal tissue with trypan blue (PHILIPPS and HAYMAN 1970), or for succinate dehydrogenase (SDH) (SMITH and GIANINAZZI-PEARSON 1990) or alkaline phosphatase (ALP) (TISSERANT et al. 1993) activities. Arbuscule frequency (A%) was also estimated and the proportion of living and functional arbuscules calculated as mentioned below:

P. brachyurus in roots was extracted using the non-destructive procedure described by Sarah (1991).

All treatments were tested in the same experiment for Smooth Cayenne and Spanish varieties however two experiments were done for Queen Tahiti variety. Each treatment consisted of 5 rep-

$$\text{Proportion of living arbuscules} = \frac{\text{A \% after staining of SDH activity}}{\text{A \% after staining with trypan blue}}$$

$$\text{Proportion of functional arbuscules} = \frac{\text{A \% after staining of ALP activity}}{\text{A \% after staining with trypan blue}}$$

licates and statistical analysis of data was performed using Newman-Keuls test following ANOVA.

Results

Both early (M) and late (1+M) inoculation with the AMF significantly increased the growth of plants compared with nonmycorrhizal controls whether they were infested or not with *P. brachyurus* (Tables 1 and 4).

Endomycorrhiza inoculation at transplanting from axenic conditions

With the single exception of Queen Tahiti variety root growth, both shoot and root growth of nonmycorrhizal microplants were significantly reduced when nematodes were introduced at the beginning of the acclimatization period. However, shoot growth of the three pineapple varieties was not significantly modified by nematode infestation in plants inoculated with the *Glomus* sp. at outplanting from axenic conditions (Table 1). Growth reductions due to the nematode were very limited and only occurred for leaf area (Spanish variety) and roots (Smooth Cayenne and Spanish varieties).

Nematode number per g. of root was significantly lower in endomycorrhizal than nonmycorrhizal roots for all three pineapple varieties (Table 1). Timing of *P. brachyurus* inoculation did not affect nematode numbers developing in the Queen Tahiti and Smooth Cayenne varieties, but later inoculation did reduce pathogen infestation in the Spanish variety (Table 1).

Nematode infestation caused a reduction in P uptake by nonmycorrhizal plants, particularly for the Smooth Cayenne and Spanish varieties, but not in endomycorrhizal plants (Table 2). For the Spanish variety, N, Ca and Mg concentrations of nonmycorrhizal plants were also decreased by nematode infestation at transplanting. However, in endomycorrhizal plants P, Ca and Mg concentrations in shoots of the three varieties inoculated with the nematodes were comparable to those of endomycorrhizal plants growing in the absence of nematodes (Table 2).

Nematode application at outplanting significantly reduced arbuscule frequency (A%) for the Queen Tahiti and Spanish varieties after trypan blue staining (Table 3) but this reduction disappeared with pathogen inoculation one month later. This showed that it was of greatest benefit to establish endomycorrhizal colonization as soon as possible. Reduction of A% was also observed after staining for SDH and ALP activities in the Queen Tahiti variety (Table 3). The proportion of living and functional arbuscules was not altered by nematode infestation (Table 3).

Endomycorrhizal inoculation at outplanting to pots

Growth of endomycorrhizal plants was significantly greater than of nonmycorrhizal plants (Table 4). Inoculation with *P. brachyurus* at outplanting reduced the growth of nonmycorrhizal but not endomycorrhizal plants (Table 4).

The nematode population was reduced in endomycorrhizal plants with both timings of the pathogen application for the Queen Tahiti variety (Table 4) but this reduction was observed for the Smooth Cayenne and Spanish varieties only when nematodes and endomycorrhizae were inoculated simultaneously. Endomycorrhiza development after nematode application did not influence nematode infestation of roots of the Smooth Cayenne and Spanish varieties. Timing of pathogen application influenced its presence in the roots of the Spanish variety; indeed the population was greater when the pathogen was introduced before inoculation with the AMF.

Nematodes negatively affected P uptake by nonmycorrhizal but not endomycorrhizal plants (Table 5). Endomycorrhiza formation enhanced Ca and Mg contents of the Queen Tahiti and Spanish varieties and N concentration for the Spanish variety. K contents were higher for the nonmycorrhizal plants.

P. brachyurus application at outplanting sig-

Table 1: Leaf area (LA), shoot (SFM) and root (RFM) fresh mass, shoot dry mass (SDM) and number of nematodes per g. of roots (Nem root) of nonmycorrhizal (NM) and endomycorrhizal pineapple at transplanting from axenic conditions (M): nematode uninoculated (Control), inoculated (Nematode) and inoculated one month later (Nematode + 1).

A – Queen Tahiti variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	186,9b	15.26b	1.76b	1.54b	0c
	M	460.7a	36.01a	4.00a	3.70a	0c
Nematode	NM	100.5c	7.35c	1.25b	0.91b	396a
	M	353.7ab	27.31ab	3.12a	3.16a	232b
Nematode+1	NM	209.0b	13.58b	1.71b	1.51b	330a
	M	444.4a	34.16a	3.96a	3.58a	250b

B – Smooth Cayenne variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	299.8c	23.35c	2.63c	2.08b	0c
	M	640.9a	54.87a	5.48a	5.02a	0c
Nematode	NM	178.6d	13.82d	1.64d	1.39c	456a
	M	540.6ab	42.86ab	3.44b	4.58a	267b
Nematode+1	NM	232.7cd	19.75c	2.11c	2.09b	418a
	M	547.5ab	48.38a	4.06b	4.64a	212b

C – Spanish variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	313.2c	21.70b	2.05c	2.25c	0d
	M	537.1a	38.48a	3.97a	3.91a	0d
Nematode	NM	96.4d	5.72c	0.75d	0.74d	350a
	M	476.9b	34.47a	2.77b	3.40ab	157b
Nematode+1	NM	263.1c	19.57b	2.26c	1.97c	180b
	M	543.6a	39.83a	3.40a	4.04a	46c

Values in a column followed by different letters are significantly different ($p<0.05$)

nificantly reduced arbuscule frequency (A%) determined after trypan blue staining in the Queen Tahiti and Spanish varieties (Table 3). A% evaluated by SDH and ALP activities was negatively affected by nematodes for the Queen Tahiti variety. However, the proportion of living and functional arbuscules was not modified by *P. brachyurus* for the three pineapple varieties (Table 3).

Discussion

Although *P. brachyurus* reduced the growth of nonmycorrhizal plants in all three pineapple varieties, in those colonized by AMF growth was not significantly affected. Precolonization of roots by AMF can therefore reduce the harmful effects of nematodes on plant growth. Simultaneous symbiont and pathogen inoculation at transplanting to pots did not affect the growth of endomycorrhizal pineapple. However, simultaneous inoculation of the AMF and nematodes at outplanting from axenic conditions slightly reduced growth of endomycorrhizal plants of the Queen Tahiti and Smooth Cayenne varieties. Both micro-organisms can be an important photosynthate sink for very young micropropagated plantlets. Effects of the nematode on young microplants of pineapple during the acclimatization period are not irreversible; indeed, late endomycorrhizal coloniza-

Table 2: Mineral concentration (% of dry mass) of shoots of nonmycorrhizal (NM) and endomycorrhizal pineapple at outplanting from axenic conditions (M): nematode uninoculated (Control), inoculated (Nematode) and inoculated one month later (Nematode + 1).

A – Queen Tahiti variety

		N	P	K	Ca	Mg
Control	NM	2.04	0.08	4.60	0.81	0.29
	M	1.70	0.18	3.59	0.96	0.37
Nematode	NM	1.65	0.08	3.64	0.82	0.28
	M	1.87	0.15	3.77	1.00	0.37
Nematode+1	NM	1.83	0.05	4.01	0.87	0.28
	M	1.77	0.18	3.49	1.04	0.37

B – Smooth Cayenne variety

		N	P	K	Ca	Mg
Control	NM	2.03	0.13	4.46	1.05	0.35
	M	1.99	0.13	4.07	1.17	0.34
Nematode	NM	1.93	0.07	4.52	1.08	0.35
	M	1.81	0.14	3.54	1.09	0.32
Nematode+1	NM	2.09	0.09	4.90	1.11	0.30
	M	1.76	0.14	3.62	1.22	0.34

C – Spanish variety

		N	P	K	Ca	Mg
Control	NM	1.88	0.13	4.13	0.75	0.26
	M	1.63	0.12	3.30	0.82	0.28
Nematode	NM	1.41	0.04	3.67	0.60	0.19
	M	1.67	0.14	3.06	0.95	0.30
Nematode+1	NM	1.82	0.09	4.06	0.89	0.26
	M	1.64	0.14	3.01	0.90	0.30

tion at transplanting to pots can compensate growth reductions of plants inoculated with nematodes at outplanting from axenic conditions. Micropropagated plantlets could tolerate better the nematode inoculation at outplanting from axenic conditions followed one month later by endomycorrhizal inoculation than both symbiotic and pathogen inoculations at the beginning of the acclimatization period. The application of nematodes at outplanting from axenic conditions without endomycorrhizal inoculation significantly reduced plant growth but this effect was not observed when plants were infested by nematodes one month later. This supports previous observations that older plants can tolerate pathogen infestation better than younger plants (COOPER and GRANDISON 1986).

Pathogen effects on endomycorrhizal colonization estimated after non vital staining varied with the pineapple variety. When nematodes were applied at transplanting this significantly reduced arbuscule frequency (A%) in the Queen Tahiti and Spanish varieties. The ability of nematodes to reduce endomycorrhizal development has also been observed by several authors (e.g. O'BANNON and NEMEC 1979, ELLIOT et al. 1984), and it has been suggested that nematodes could induce an unfavourable environment for infection by the fungal symbiont (THOMAS et al. 1989). Although nematode infestation negatively influenced val-

Table 3: Arbuscular frequency observed after staining with trypan blue (TB), succinate dehydrogenase (SDH) or alkaline phosphatase activities and proportion of living (SDH/TB) and functional (ALP/TB) arbuscules of endomycorrhizal pineapple roots at outplanting from axenic conditions (M) and at transplanting to pots (1+M): nematode uninoculated (Control), inoculated (Nematode) and inoculated one month later (Nematode+1).

		Frequency of arbuscules (A%) detected by staining for				
		Total fungal tissue (TB)	SDH activity (%)	SDH/TB	ALB activity (%)	ALB/TB
Queen Tahiti variety						
M	Control	63a	24.5a	0.39a	16.4a	0.26a
	Nematode	46b	14.7b	0.32a	9.4b	0.21a
	Nematode+1	56ab	17.4b	0.31a	12.0b	0.22a
1+M	Control	65a	19.0a	0.29a	12.7a	0.20a
	Nematode	33b	12.7b	0.38a	8.0b	0.24a
	Nematode+1	38b	13.6b	0.36a	9.2b	0.24a
Smooth Cayenne variety						
M	Control	65a	20.3a	0.31a	15.8a	0.24a
	Nematode	50a	20.0a	0.40a	13.9a	0.27a
	Nematode+1	51a	17.5a	0.34a	14.0a	0.27a
1+M	Control	53a	13.8a	0.26a	11.0a	0.21a
	Nematode	60a	10.9a	0.18a	8.0a	0.13a
	Nematode+1	68a	14.9a	0.22a	10.3a	0.15a
Spanish variety						
M	Control	50a	21.6a	0.43a	13.8a	0.28a
	Nematode	29b	16.5a	0.56a	10.2a	0.35a
	Nematode+1	38ab	18.6a	0.49a	10.0a	0.27a
1+M	Control	40a	18.6a	0.46a	12.2a	0.30a
	Nematode	31b	16.7a	0.54a	9.4a	0.30a
	Nematode+1	35ab	14.4a	0.41a	9.4a	0.27a

Values for each combination of variety and inoculation treatments (nematode and endomycorrhizal fungus) followed by different letters are significantly different ($p<0.05$)

ues for A% of Queen Tahiti variety estimated by SDH and ALP activities, *P. brachyurus* did not affect the proportion of living and functional arbuscules of the three pineapple varieties, and consequently did not influence the efficiency of the symbiosis for pineapple. This could partly explain the lack of effect of *P. brachyurus* on the growth of endomycorrhizal plants.

Several reports have shown that endomycorrhizal colonization decreases nematode populations in root systems (e.g. BAGYARAJ et al. 1979, SALEH and SIKORA 1984), and SMITH et al. (1986) showed that AMF can enhance plant tolerance to nematodes in field conditions. In this study, numbers of nematodes were also significantly reduced in the roots of endomycorrhizal pineapple of the three varieties in comparison to nonmycorrhizal plants, whether nematodes were applied simultaneously with or after the AMF. Reductions in nematode infection have been attributed to modifications in plant physiology caused by the symbiotic fungi. AMF are able to ensure an adequate P nutrition in presence of nematodes and since P is considered as an important factor in plant tolerance (SMITH and KAPLAN 1988), higher concentrations of this element in endomycorrhizal tissues could have a direct action reducing nematode numbers in roots (MACGUIDWIN et al. 1985). Changes in root exudates may also alter root attractiveness for nematodes (MACGUIDWIN et al. 1985), or induce physical and chemical barriers to root penetration (KELLAM and SCHENCK 1980).

Table 4: Leaf area (LA), shoot (SFM) and root (RFM) fresh mass, shoot dry mass (SDM) and number of nematodes per g. of roots (Nem root) of nonmycorrhizal (NM) and endomycorrhizal pineapple at transplanting to pot (1+M): nematode uninoculated (Control), inoculated (Nematode) and inoculated one month later (Nematode+1).

A – Queen Tahiti variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	312.4b	24.82b	2.52b	2.58b	0c
	1+M	452.9a	33.85a	3.19a	3.36a	0c
Nematode	NM	136.9c	10.95c	1.19c	1.13c	564a
	1+M	389.5ab	31.62a	2.74ab	3.00a	300b
Nematode+1	NM	176.7c	13.11c	1.26c	1.28c	465a
	1+M	433.8a	35.26a	2.77ab	3.53a	304b

B – Smooth Cayenne variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	299.8b	23.35b	2.63b	2.08b	0c
	1+M	471.7a	34.65a	3.06a	3.07a	0c
Nematode	NM	178.6c	13.82c	1.64c	1.39c	456a
	1+M	362.3ab	31.24a	2.84a	3.65a	389a
Nematode+1	NM	232.7bc	19.75b	2.11b	2.09b	418a
	1+M	485.1a	35.47a	3.05a	3.85a	293b

C – Spanish variety

		LA (cm^2)	SFM (g)	RFM (g)	SDM (g)	Nem. root
Control	NM	313.2b	21.70b	2.05b	2.25b	0d
	1+M	483.5a	33.45a	2.92a	3.23a	0d
Nematode	NM	96.4c	5.72c	0.75c	0.74c	350a
	1+M	465.0a	33.68a	2.29ab	3.40a	330a
Nematode+1	NM	263.1b	19.57b	2.26ab	1.97b	180b
	1+M	432.7a	31.29a	2.65a	2.85a	122c

Values in a column followed by different letters are significantly different ($p<0.05$)

Endomycorrhizal colonization could also represent a competition for photosynthates in roots (SMITH 1987), and thus produce a less favourable environment for the nematodes (KELLAM and SCHENCK 1980) or influence the quality of food reserves of nematodes (MACGUIDWIN et al. 1985). Endomycorrhizal plants have higher sugar contents, modified hormone balance and modifications in the composition of amino acids (e.g increases in serine and phenylalanine which are nematicidal) (SURESH et al. 1985). Presence of a fungal symbiont in roots can affect the normal life cycle of nematodes (CASON et al. 1983) and reduce nematode size (SITARAMAIAH and SIKORA 1982).

Other micro-organisms such as bacteria and fungi are also considered as antagonists to nematodes (CAYROL et al. 1992), and the combination of AMF with one or several antagonists could produce a more beneficial synergistic action on plant protection and growth. Fallow could be also used to combat nematode populations in soil (STIRLING and NIKULIN 1993), but this approach risks decreasing endomycorrhizal potential and reducing soil fertility (SARAH 1987) unless inoculation with efficient AMF after fallow is ensured. The control of nematodes in pineapple, which avoids excess use of nematicides, clearly requires an integrated approach. The results reported here suggest that endomycorrhizae, which are not affect-

Table 5: Mineral concentration (% of dry mass) of shoots of nonmycorrhizal (NM) and endomycorrhizal pineapple at transplanting to pots (1+M): nematode uninoculated (Control), inoculated (Nematode) and inoculated one month later (Nematode+1).

A – Queen Tahiti variety

		N	P	K	Ca	Mg
Control	NM	1.72	0.10	3.83	0.83	0.33
	1+M	1.76	0.17	3.74	0.79	0.33
Nematode	NM	1.83	0.05	4.60	0.67	0.24
	1+M	1.95	0.13	3.91	0.88	0.34
Nematode+1	NM	2.22	0.08	5.25	0.74	0.27
	1+M	1.81	0.12	3.42	0.91	0.32

B – Smooth Cayenne variety

		N	P	K	Ca	Mg
Control	NM	2.03	0.13	4.46	1.05	0.35
	1+M	2.26	0.16	4.46	1.18	0.33
Nematode	NM	1.93	0.07	4.52	1.08	0.35
	1+M	1.70	0.16	3.38	1.03	0.33
Nematode+1	NM	2.09	0.09	4.90	1.11	0.30
	1+M	1.85	0.16	3.68	1.13	0.35

C – Spanish variety

		N	P	K	Ca	Mg
Control	NM	1.88	0.13	4.13	0.75	0.26
	1+M	1.97	0.16	4.01	0.83	0.27
Nematode	NM	1.41	0.04	3.67	0.60	0.19
	1+M	1.74	0.13	3.18	0.83	0.26
Nematode+1	NM	1.82	0.09	4.06	0.89	0.26
	1+M	1.98	0.14	3.98	0.91	0.27

ed by nematicides (HABTE and MANJUNATH 1988), could be a valuable component in a scheme of integrated protection against nematodes.

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SELOSTUS

Arbuskelimykorritsasienten käyttö *Pratylenchus brachyurus* -ankeroisen torjunnassa mikrolisätyllä ananaksella

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Ananaksen juuristosta tavattua ankeroista *Pratylenchus brachyrus* pidetään merkittävänä taudinaiheuttajana ananasviljelyksillä. Mikrolisättyihin ananaslajikkeisiin 'Queen Tahiti', 'Smooth Cayenne' ja 'Spanish' siirrostettiin *Glomus*-mykorritsasientä ja/tai ne tartutettiin *P. brachyrus* -ankeroisella. Siirrostus ja tartutus suoritettiin välittömästi ananaksen *in vitro* -vaiheen jälkeen tai kuukautta myöhemmin. Ankeroinen ei haitannut mykorritsallisten taimien kasvua. Ankeroisen aikainen tartutus heikensi mykorritsattomien taimien kasvua mutta myöhäinen tartutus ei. Mykorritsan ansiosta ankeroisten lukumäärä/juurigramma väheni ankeroistartutuksen ajankohdasta riippumatta. Ankeroiset vähensivät merkittävästi mykorritsasienen kokonaisinfektiota lajikkeissa 'Queen Tahiti' ja 'Spanish' mutta eivät vaikuttaneet metabolisesti aktiivisten arbuskeleiden kehitykseen tutkittujen kolmen lajikkeen juuristossa. Mykorritsasiirrostus lisäsi kasvien versojen fosforipitoisuutta. Aikainen ankeroistartutus vähensi hiukan mykorritsattomien kasvien kivennäispitoisuuksia.