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Foliar calcium concentration of potato and its relation to genotype lateness and tolerance of cyst nematodes

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Abstract

In field trials during three years respectively 18, 22 and 57 potato genotypes were grown on soils moderately or heavily infested with potato cyst nematodes (*Globodera pallida)* after soils were fumigated or not. Nematode infection increased leaf calcium contents but genotypes that were relatively tolerant of potato cyst nematodes (*Globodera pallida)* had lower leaf calcium concentrations on a particular sampling date. Tolerance of potato cyst nematodes was closely linked to genotype lateness and may be related to tolerance of drought. When using the method in plant breeding and screening for genotypes with tolerance, plants may be planted in infected or in uninfected soils, without influencing the outcome. The relationship between tolerance and calcium contents was clearest when sampling took place towards the end of the growing season when the variability was at it highest with a good distinction between newly formed leaves in late maturing genotypes and aged leaves in early maturing cultivars.

Introduction

Trudgill et al. [1975] reported a decrease of the N and P and an increase of Ca and Na concentrations in the leaves of potato following infection with potato cyst nematodes. Especially P and K concentrations (based on dry weight) were reduced from 0.43 to 0.30 mg g^{-1} P and from 6.4 to 3.5 g mg g^{-1} K going from a light to a heavy infestation, meanwhile the Ca concentration increased from 1.2 to 2.7 and the Na concentration from 0.011 to 0.025 mg g^{-1} foliage.

The calcium concentration in plant parts is decreased by water stress. This because the plant takes up less water per unit dry matter production and calcium is transported with the transpiration flow. Supply of calcium to plant roots can be accounted for by mass flow in most soils [Brewster and Tinker, 1970]. It has been shown that potato cyst nematodes reduce the water uptake of potato plants [Evans, 1982; Haverkort et al., 1991] so one would expect potato cyst nematodes to reduce the calcium concentration. There has been found, however, a positive correlation between the population density of potato cyst nematode and the calcium concentration of the leaves [e.g. Evans and Franco, 1979; Evans, 1982]. Two hypotheses [Fatemy and Evans, 1986] may explain the increased uptake of calcium following nematode attack. Firstly, Price and Sanderson [1984] found that oat roots containing *Heterodera avenae* juveniles translocated over eleven times the amount of uninfected control roots; they suggested that nematode invasion damaged the endodermis and maintained an apoplastic pathway for the uptake of Ca in regions of the roots where such a pathway normally is unoperative (i.e. where Casparian bands and secondary endodermal thickening are present). Secondly, infected plants may have taken up more Ca to maintain their ionic balance because nematode infection reduced uptake of the cations (e.g. K) which are taken up actively [Mengel and Kirkby, 1978].

Table 1. Details of the field trials

	Year		
	1990	1991	1992
Planting date	April 17	April 17	April 15
Site	Eeserveen	Smilde	Annerveenschekanaal
Number of genotypes	18	22	57
Number of replicates	2	4	3
Harvested number of plants per (periodic) harvest	30	20	12
Soil pH	4.7	4.5	4.4
Soil% organic matter	5.9	12	28
Number of living eggs per g soil			
before fumigation	53	41.4	13.4
after fumigation	6	11.0	3.2
Date of sampling for Ca			
T1	July 18	June 21	July 17
T2		July 29	August 18
Plant part sampled	haulms	young leaves	young leaves
Harvest date	October 10	6 October	12 October

Evans and Franco [1979] correlated the calcium content of ten different cultivars to their tolerance of potato cyst nematode and suggested that differences in nematode tolerance may have been due to differences in water use. Genotypes that more effciently use water and have lower calcium concentrations, it was hypothesized, would be more tolerant of potato cyst nematodes. Therefore, Evans [1982] tested a range of cultivars in the field and in pots, either or not infected with potato cyst nematodes *(Globodera rostochiensis)* and determined the calcium concentration of the complete haulms (mean value) or sampled the fifth leaf 80 days after planting . He found that ranking the cultivars according to their tolerance gave a different order per site and year than when ranking according to calcium concentration of the firth leaf. Moreover, with early cultivars, the calcium concentration of the fifth leaf corresponded well with that of the complete haulms but with late cultivars the fifth leaf had lower concentrations indicating that such leaves were younger, had transpired less water in their lifetime and had accumulated less calcium. A large percentage of calcium in the leaves means that they have stopped growing but are still transpiring. Correlating water-use efficiency and tolerance would only be valid when genotypes of the same maturity are used or when the calcium uptake by the whole plant is considered. Here the same difficulty in interpreting data exists as was found by Haverkort and Valkenburg [1992] on the discrimination of ^{13}C which, beside water-use efficiency, correlates well with the the degree of infection with potato cyst nematode and the age of the plant.

It has been suggested by authors such as Trudgill et al. [1990] and Haverkort et al. [1992] that late cultivars automatically are more tolerant of potato cyst nematodes because they invest more dry matter in the foliage. Late cultivars arrive at higher leaf area index values and consequently the reduction of total dry matter production following nematode attack is less than with early cultivars. Such early cultivars only reach LAI values of 3–4 and hence after infection they more easily arrive at light interception values less than 100% than late maturing cultivars. Nematodes reduce yields through a number of mechanisms: initially hormonal signalling [ABA according to Evans, 1982] and subsequently reduced water uptake [Haverkort et al., 1991] leads to stomatal closure reducing photosynthesis; finally reduced uptake of nutrients, especially phosporus or nitrogen [Trudgill, 1980] reduces the efficiency of assimilation processes and leads to a decreased dry matter accumulation. Phosphorus uptake depends on root growth, distribution and uptake capacity, whereas the uptake of cations such as Na, K and Ca follows mass water flows through the soil towards the roots.

The objectives of the present research were to find out whether tolerance of potato cyst nematode is related to foliar calcium concentrations and lateness of the cultivar and whether tolerance of nematodes is related to tolerance of drought. If the correlations are sufficiently good, the technique might offer scope as a screening technique to be used in plant breeding. To investigate this, during three years field trials were carried out with many cultivars and final fresh tuber yields were correlated to leaf calcium concentrations before plant senescence.

Materials and methods

Field trials on light sandy peat soils with pH values of about 4.5 were carried out in 1990, 1991 and 1992. Details of the experiments are given in Table 1. The fields were naturally infested with *Globodera pallida*. The experiments were designed as split plots. The planting pattern was 75 cm between the rows (plots were four rows wide) and 30 cm between the plants within the row. The two central rows were harvested and between the periodic harvests in the experiments in 1991 and 1992 two plants within the row were left as guards. The number of plants harvested per (periodic) harvest is shown in Table 1. Half of each plot was fumigated with metham sodium (500 l per ha, 510 g active and ingredient per litre), applied a few weeks before planting with a spade injector. Each sub-plot was planted with a different genotype and the planting pattern was 75 cm × 30 cm. NPK fertilizers were applied according to standard recommendations for starch potato production following soil sampling in early spring resulting in 200 kg ha^{-1} N, 250 kg $ha^{-1} P_2O_5$ and 300 kg ha^{-1} K_2O available to the crop at planting. The seed tubers were graded in 35–45 mm sizes and were treated against *Rhizoctonia solani* with validamicine. The crops were irrigated overhead to avoid drought stress. When sampling the crops for leaf calcium content, in 1990 an average sample of the complete tops was taken and in the two subsequent years a few of the youngest fully grown leaves were collected. The leaves or haulms were dried overnight at 105 °C and extracted with TCA/HCl before analysis with an atomic absorption spectrophotometer (SpectrAA-1-, Varian Techtron, Australia). The genotypes used (cultivated varieties and advanced breeding clones) varied from early to late and from intolerant to tolerant of potato cyst nematodes. The experimental details particular to each field trials are shown in Table 1. Earliness rating of cultivars was taken from the Netherlands Recommended List of Cultivars.

Results and discussion

Table 2 shows the average tuber yields of all genotypes, the highest and lowest yield of a genotype and the least significant difference between any treatment (fumigation and genotype combination) per experiment. The extremely intolerant genotype marked 1 in Figure 1 is not included in Table 1.

Figure 1 shows the calcium concentrations of the leaves of the genotypes in the three field trials. In 1990, for example, 18 genotypes were grown on fumigated and unfumigated soil. The proportion of the yield of each individual genotype obtained in unfumigated soil compared to fumigated soil (relative yield) is plotted on the ordinates in both Figures 1A and B. In Figure 1A these relative yields are plotted versus the leaf calcium concentration of the 18 fumigated plots (on the abscissa) and in Figure 1B the same relative yields are plotted versus the leaf calcium concentration of the unfumigated plots. The calcium concentration varied widely between the experiments indicative of a site and/or site × year interaction. Figure 1 shows the variation to range between 1.3 and 3.5% in 1990, between 1.3 and 1.9% in 1991 and between 1.6 and 3.5% in 1992. That calcium concentrations were so much lower in 1991 than in 1990 may be due to the 1991 temperature sum and cumulative amount of global solar radiation between May 1 and July 15 that were about 25% less in 1991 than in 1990 (data not shown). Consequently the evaporative demand was much lower in 1991 than in 1990 (and 1992) and the leaves accumulated much less calcium. In 1990 whole haulm was sampled and in the two other years only the youngest fully grown leaves which may have contributed to the differences found between the two years. But not fully as in 1992 also higher calcium concentrations than in 1991 were found in leaves. Figure 1 also shows that relatively good correlations existed between relative yields and leaf calcium content in 1990 and 1991 (r^2>0.35) but not in 1992 (r^2<0.1). The field planted with potato in the 1992 trials had a relatively low infection rate of only 13 eggs per g soil before and of 3 after fumigation. This difference was much less than in the two preceding years which may well explain the lack of response in the 1992 trial: the relative yields ranged between 0.5 and 1.0 in 1992 versus 0.25 and 1.0 in 1990.

The relationships found in Figure 1 confirm the finding of Evans [1982] that high levels of calcium in the leaves are associated with low levels of tolerance. The relationships hold whether Ca-levels are deter-

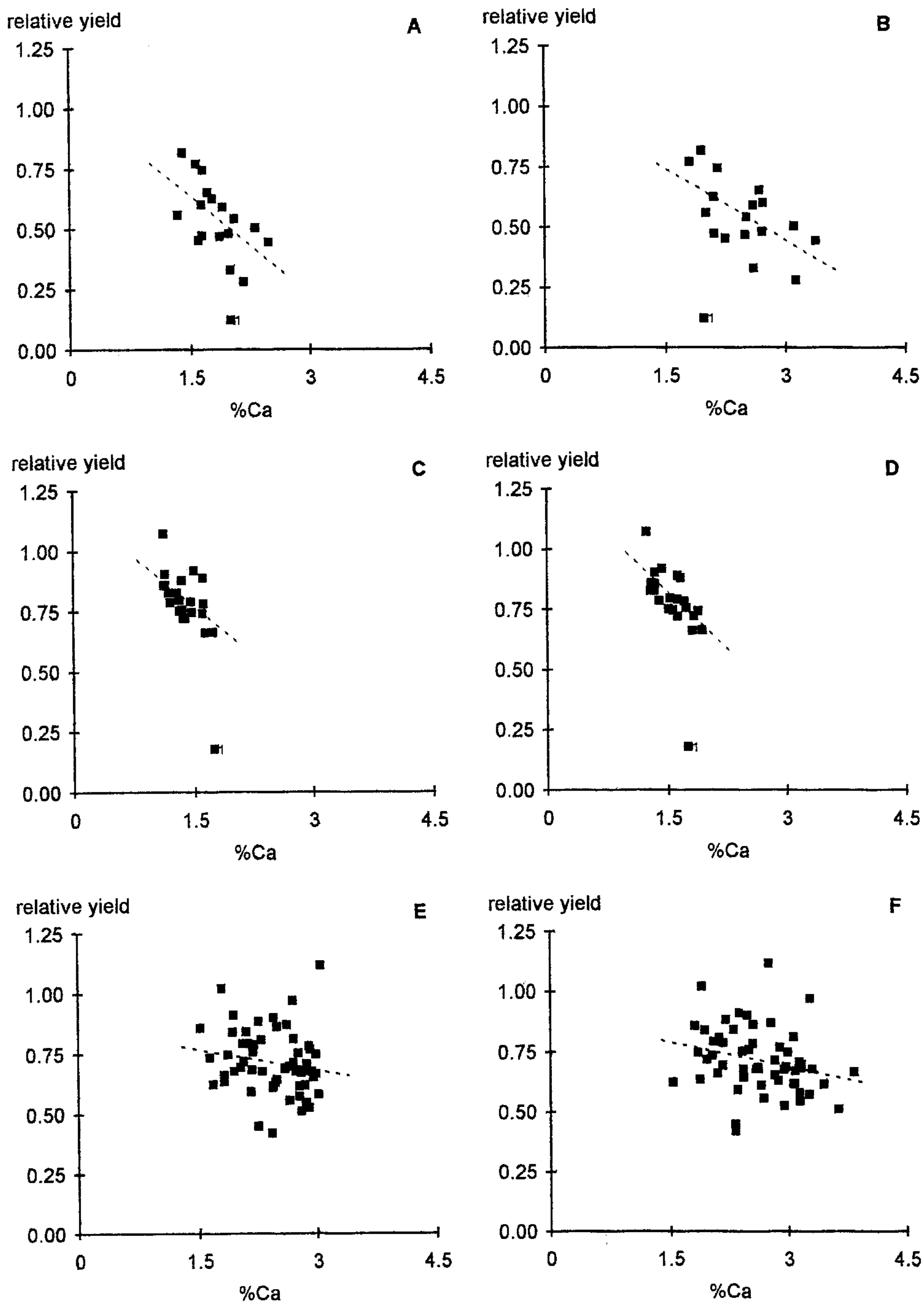

Figure 1. Relative fresh tuber yields (1.00 equals yield of a genotype on fumigated plots) in the field trials in 1990 (A,B), 1991 (C,D) and 1992 (E,F) versus leaf calcium concentration of plants grown on fumigated (A,C,E) and unfumigated (B,D,F) plots. Data points marked with 1 represent relative yields of extremely intolerant clones (not cultivars) and are not included in the regression line. Mean [Ca] (%): A: 1.84, B: 2.45, $LSD_{0.01}$ between A and B: 0.19 C: 1.38, D: 1.56, $LSD_{0.01}$ between C and D: 0.08 E: 2.45, F: 2.61, $LSD_{0.01}$ between E and F: 0.11

Table 2. Tuber fresh matter yields (g m^{-2}) at crop senescence

		Experiment		
		1990	1991	1992
Average tuber yield	– fumigated	4668	4283	6059
	– unfumigated	2444	3348	4326
Highest yielding genotype	– fumigated	5650	5948	8807
	– unfumigated	3826	5111	7681
Lowest yielding genotype	– fumigated	4148	3382	3000
	– unfumigated	566	2412	1607
Least Significant Difference	(P = 0.01)	310	295	299

mined in the plots that are heavily (unfumigated) or lightly (fumigated) infected: the response is the same although the average calcium concentration was higher in the infected plots: from 1.84% tot 2.45% in 1990, from 1.38 to 1.56% in 1991 and from 2.50 tot 2.62 in 1992.

Figure 2 shows the relationship between calcium concentration and the earliness of the genotypes used as shown in the Netherlands Recommended List of Varieties: 2=very late maturing cultivar and 10=very early maturing cultivar. Lateness of a cultivar clearly is associated with lower calcium concentrations. This corroborates the finding of Evans: early cultivars have determinate top growth and at maturity all assimilates are diverted into the tubers. Hence, as the the tuber calcium concentration is lower than those of the leaves, the calcium taken up in the transpiration stream accumulates in the leaves. Conversely, late maturing cultivars continue to produce new leaves and, consequently the amount of water transpired (and hence Ca^{2+} accumulated) by a unit of leaf is less than for early cultivars. The presence of potato cyst nematodes increases the calcium concentration (Figure 2A) but not more than proportionately so there is no interaction between lateness and infection with potato cyst nematode. As was shown in Figure 1, it does not seem to matter whether infected or uninfected plants are taken to be ranked according to lateness and or tolerance. Choosing the right moment of sampling during the growing season is of greater importance when relating tolerance to leaf calcium content than the choice between sampling either infected or uninfected plots. Figs. 2B and 2C regress the earliness of genotypes according to the Netherlands List of Recommended Varieties grown on fumigated plots versus leaf calcium concentrations. Early in the season leaves are relatively young, have transpired relatively little water and thus have low calcium concentrations. Later in the season, average calcium concentrations have increased, and the relationship between calcium concentration and percentage yield loss becomes more marked. This conclusion comes from the two Figs. 1 and 2 combined. Figure 1 shows decreased relative yields at higher calcium concentrations and Figure 2 B and C shows that genotypes differ in concentration when crops are older: hence later sampling shows a better relation between relative yield losses (tolerance) and leaf calcium concentrations. Figs. 2B and 2C show that the lines of calcium concentrations on the first harvest dates (triangles) have a smaller slope than those of the second harvest (quadrangles). Choosing the right moment means not too early because no differences will have built up and not too late because the earliest cultivar still need to have green leaves to be sampled. From this it appears that to establish relationships between tolerance of potato cyst nematodes, plants should be sampled relatively late in the growing season. In the Figs. 1 E and F no relationship between Ca concentration and tolerance could be demonstrated in the 1992 trial because the infection pressure was too low. In the 1992 trial, however, the leaf calcium concentrations correlated well with cultivar lateness, as in 1991, especially when sampling took place late in the growing season (late August early September). The association of cultivar lateness and tolerance of potato cyst nematodes can also be inferred from data provided by Trudgill and Phillips [1994] indicating that rates of canopy closure, associated with vigour of top growth, are major factors influencing tolerance differences.

Plotting the relative tuber yields (ratio tuber yields with low and with high numbers of potato cyst nema-

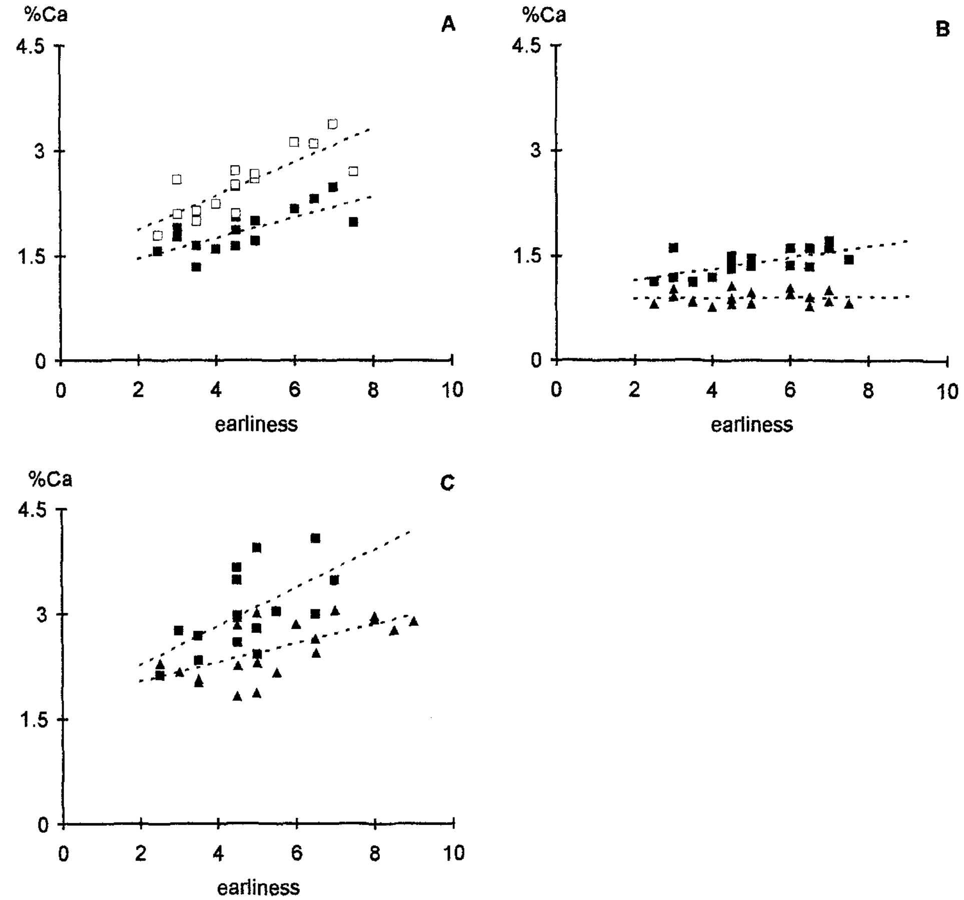

Figure 2. Relationships between calcium concentration and the earliness of genotypes according to the Netherlands List of Recommended Varieties: 2=very late, 10 = very early. A: sampling date July 18 1990, (open symbols unfumigated, closed symbols fumigated), B: 1991 (fumigated) sampling date triangles June 21, rectangles July 29, C: 1992 (fumigated) sampling date triangles July 17, rectangles August 18., r^2 A>0.53, r^2B>0.43, r^2 C>0.37.

todes) of the three years versus lateness (Figure 3 A,C,E) shows that the latest cultivars on average gave the highest relative yields, and, apparently, were least affected by the presence of nematodes. This effect of lateness on tolerance was especially noticeable in 1990 and least so in 1992. The relative tuber yields of cultivars differing in earliness varied more widely at the final harvest than at the harvest carried out at the foliar maximum (Figure 3A). This is explained because later cultivars have more time available at the end of the growing season when the yield accumulation of early cultivars has ceased.

It has been hypothesized before that tolerance of abiotic (drought) and of biotic (potato cyst nematodes) stress should be linked through genotype lateness [Haverkort et al., 1992. The later a cultivar is, the later its tubers are initiated [Kooman and Haverkort, 1995] and the more foliage is formed. When subjected to a stress factor (drought, nematodes), it was shown before [Trudgill et al., 1990 and Haverkort et al., 1992] that light interception (leave shedding leading to a reduced leaf area index and light interception) is reduced more than the light use efficiency. Later cultivars show reduced light interception to a lesser extent than early cultivars as during a greater part of the growing season their LAI-values are above 3 meaning still 100% light interception. This effect became apparent in 1990 and 1991 (Figure 3B,D): higher ratings for drought tolerance in the Netherlands Recommended List of Cultivars were also associated with relatively higher yields in the presence of nematodes. The range of drought tolerance present in the cultivars was not broad, however, (rates of 6, 7 and 8 only) and the variation was considerable. In the experiment of

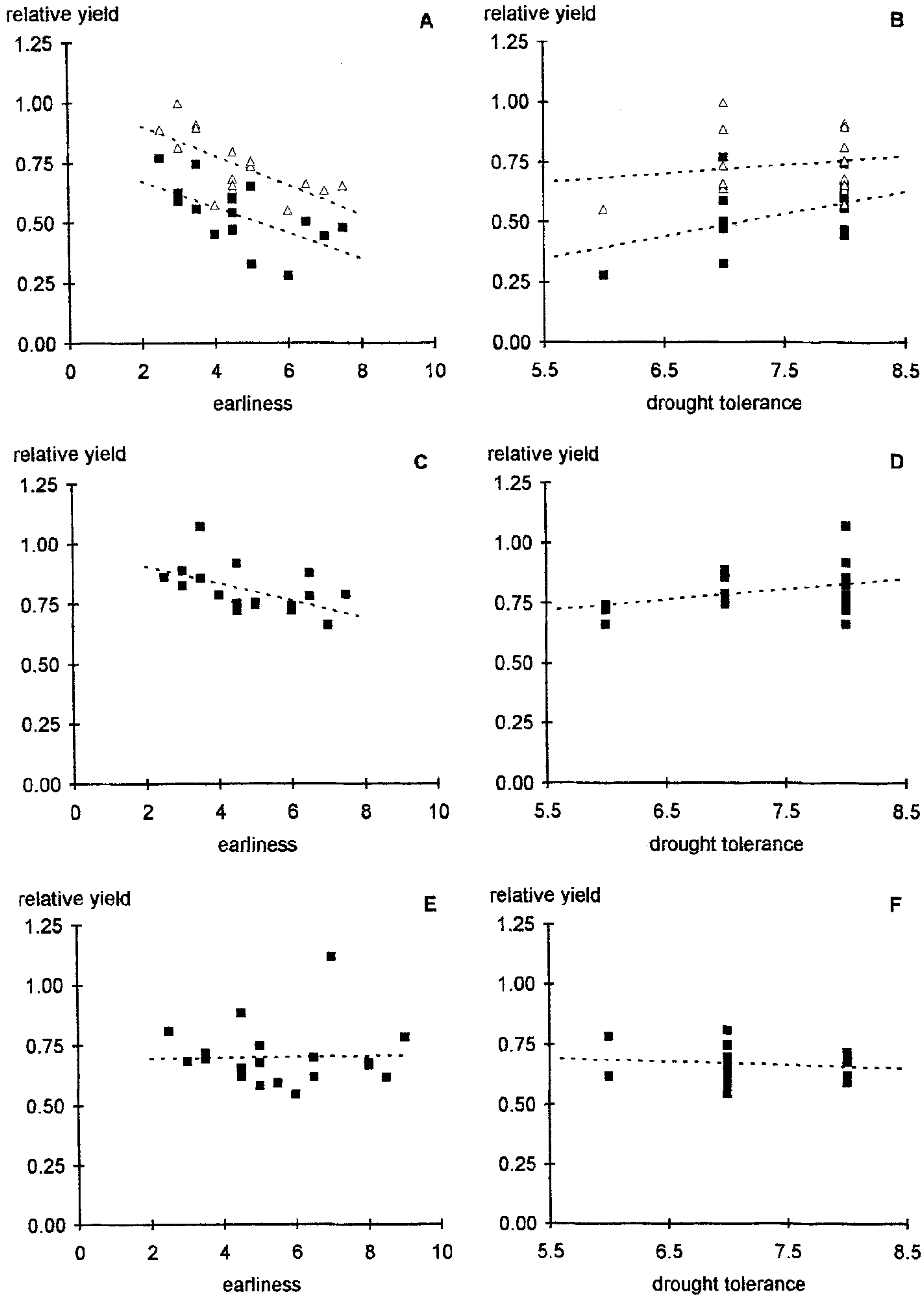

Figure 3. Relationships between relative tuber yields (unfumigated/fumigated) at crop senescence (closed symbols) in the experiments in 1990 (AB), 1991 (CD) and 1992 (EF), and cultivar earliness (A,C,E, 2=very late and 10 =very early) and drought tolerance (B,C,D, 5=intolerant, 9=tolerant) according to the Netherlands Recommended List. The open symbols in Figs 3A and 3B represent the relative tuber yields at the foliar maximum) r^2 A and C>0.31, r^2 B and D >0.19, r^2 E and F <0.02.

1992 (Figure 3F), the response of relative tuber yields following potato cyst nematode infection, as with earliness (Figure 3E) was not present (Figure 3F), probably because of the small difference of infection levels of the soil with and without fumigation.

Conclusions

The results of the experiments presented here corroborate those of Evans [1982]. Nematode infection leads to increased leaf calcium contents. Genotypes that are relatively tolerant of potato cyst nematodes (*Globodera* pallida) have lower leaf calcium concentrations on a particular sampling date. In the present research we established the relationship between tolerance and leaf calcium content under field conditions for a wide range of genotypes in three different planting seasons. Moreover, it was shown that tolerance of potato cyst nematodes is closely linked to genotype lateness and we indicated that lateness may also be linked to tolerance of drought (as shown from Recommended List data). The finding of Cotes et al. [1981] that nematode control is more effective in early cultivars than in late ones is in line with our findings. It was shown that when using the relation between leaf calcium concentration and tolerance and lateness in plant breeding (when screening for genotypes with tolerance) plants may be planted in infected or in uninfected soils, without influencing the outcome. Furthermore, it is recommended to sample the latest formed fully grown leaves towards the end of the growing season, as then the variability is at it highest with a good distinction between newly formed leaves in late maturing genotypes and aged leaves in early maturing cultivars.

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