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The influence of cyst nematodes and drought on potato growth. 3. Effects on carbon isotope fractionation

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Abstract

A good agreement was found between drought induced water-use efficiencies and carbon isotope fractionation of potato plants of the same age. Discrimination of ^{13}Carbon isotope increased with plant age and with the rank order of the distinguished plant organs. In young plants, nematode infection (contrary to drought) reduced ^{13}C discrimination but also reduced the water-use efficiency, leading to the conclusion that nematode infection, by slowing down the plant metabolism may have reduced the extrusion of ^{13}C. Towards senescence both nematode infection and drought reduced ^{13}C discrimination and increased water-use efficiencies indicating that then nematode caused water stress in the plants.

Additional key words: Globodera pallida, Solanum tuberosum L., water-use efficiency

Introduction

Higher plants with conventional (C3) pathways have a $^{13}C/^{12}C$ ratio about 20 ‰ less than in the atmosphere (Farquhar et al.,1982). C3 plants discriminate against ^{13}C during diffusion from the air to the intercellular spaces, and by the primary carboxylation enzyme ribulose 1,5 biphosphate carboxylase. Farquhar et al. (1982) showed that with a decrease of photosynthesis, which leads to higher values of the intercellular/ambient CO_2 concentration ratios (C_i/C_a), discrimination against ^{13}C is greater. Hence, discrimination will be greatest at the light compensation point (when C_i/C_a equals unity) and least at light saturation. If, however, plant photosynthesis decreases due to an increase of the stomatal diffusion resistance (in the case of water stress for instance), then the C_i/C_a ratio decreases and discrimination is less, because more ^{13}C accumulates in the stomatal and intercellular cavities, and more ^{13}C is carboxylated.

Air has a -8.5 ‰ ^{13}C isotope composition relative to the PDB standard whereas Farquhar et al. (1982) showed that plants on average had values of about -30 - -22 ‰. Respired CO_2 was up to 8 ‰ richer in ^{13}C than the plant.

Farquhar et al. (1982) indicated that dark respiration, photorespiration and environmental factors such as light, temperature, salinity and drought all mainly affect discrimination via their effect on the intercellular CO_2-concentration. Farquhar and Richards (1984) found smaller isotope effects (less discrimination) in winter wheat that suffered from drought and a good linear relationship between water-use efficiency and the isotope effect. Discrimination was less in the grain than in the stems and in the leaves, but there were high correlations in ^{13}C content between the different plant parts.

Until yields were half that of well fertilized soils, going from very severe nutrient stress to non limiting conditions, the water-use efficiency increased with increased nutrient (NPK) availability (Tanner and Sinclair, 1983). Hubick et al. (1986) found differences in water-use efficiencies up to to 60 % in water stressed peanuts which correlated well with the isotope effect ($r = -0.81$).

The 35 % remaining variation in the relation was explained by these authors in terms of several effects. Droughted plants transpire less and have a higher leaf temperature. Farquhar et al. (1982) showed that ^{13}C discrimination increased with about 1.2 ‰ per °C. Hubick et al. (1986) used leaves instead of a sample of the whole plant, they suggested that the water-use efficiency of the whole plant probably correlated better with the isotope effect of the whole plant. Vos and Groenwold (1989) reported a good correlation between ^{13}C isotope fractionation and water-use efficiencies of 6 potato cultivars.They argued that carbon isotope analysis shows potential as an indirect screening method to identify genetic differences in water-use efficiency.

The objective of the work presented here is to determine the effect of potato cyst nematode infection and drought on carbon isotope fractionation. Haverkort et al. (1991) showed that young infected plants, contrary to droughted or old infected plants, had lower water-use efficiencies than uninfected well watered plants. Carbon isotope analysis may help elucidate the mechanismes behind these observed differences.

Materials and methods

Six potato plants cv. Mentor were grown in containers and subjected to combinations of the following treatments: with and without on average 18.5 juveniles of *Globodera pallida* per gram soil and either well watered throughout the season, subjected to drought during the first 43 days after planting or subjected to drought from 43 days after planting until senescence at 92 days after planting. Three periodic harvests were made: on 43, 70 and 92 days after planting. The water-use efficiency (dry matter weight produced per litre water used) was calculated at each harvest date, based on the total amount of dry matter produced from planting till harvest, and the amount of water supplied to the container in the same period. To accomodate for leaf shedding, total dry matter weight at maturity was considered to consist of the total dry weight of the tops at the foliar maximum at the second harvest and the tuber dry matter at maturity. A slight overestimation may then take place because of a relocation of dry matter from the stems to the tubers, but using observed haulm dry matter at the final harvest would have led to an underestimation of total dry matter produced. The first of the three periodic harvests, in which all of the six plants per container were lifted, took place at the end of the early drought period (43 days after planting) which was also the start of the late drought stress period. In the middle of the late drought period a second harvest was made at 70 days and the final harvest at maturity was at 92 days after planting. The experiment, of which full details are given by Fasan and Haverkort (1991) was laid out in a complete bloc design in three replicates in which the containers of the two nematode treatments, the three drought treatments and the three harvest dates were completely randomized.

About 3 mg of finely ground samples of dry matter of each plant part from each periodic harvest was combusted in pure oxygen at 1000 °C. Combustion gasses were cleansed with Cr_2O_3 and Ag and further oxidized during passage through a furnace containing CuO pellets at 900 °C. Nitrogenous compounds were reduced to N_2 in a tube with Cu pellets at 600 °C. Water was trapped in a tube filled with $Mg(ClO_4)_2$ powder. The resulting mixture of N_2 and CO_2 was separated through gas chromatography (carrier gas: Helium) at temperatures between 75 and 125 °C. The mass spectrometer used (Europe Scientific Ltd., Crewe, UK) contains a triple collector which distinguishes between atomic masses of 44 ($^{12}C^{16}O^{16}O$), 45 ($^{13}C^{16}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$). The results were expressed in terms of the standard PDB defined by:

$$^{13}\delta = (^{13}R_{\mathrm{p}} / {}^{13}R_{\mathrm{PDB}} - 1) \times 1000\ (‰)$$

where $^{13}R_{\mathrm{p}}$ is the molar abundance ratio $^{13}C/^{12}C$ of a sample. Following established conventions, in this paper, fractionation of ^{13}C ($^{13}\epsilon$) is reported.

The value of $^{13}\epsilon$ (‰) is defined as the ^{13}C content of the plant material relative to the ^{13}C content of the atmosphere, $^{13}\delta_{\mathrm{PDB}}$ of atmospheric CO_2 set at -8.5 ‰). Vos and Groenwold (1989) showed how $^{13}\epsilon$ of a sample is calculated from its $^{13}\delta$ value.

As a rule, one sample was analyzed per replicate. The error of the spectrometer varied from 0.015 to 0.025 % in two test in which 6 subsamples of a larger sample were analyzed. The coefficient of variation of $^{13}\delta$ values per treatment per plant part per harvest varied from 1 to 4 %.

Results

The green leaves showed the lowest $^{13}\epsilon$ values of all plant parts at all three harvests (Fig. 1). Yellow leaves, recorded only at the second harvest, on average had 0.5 ‰ higher values. Stems, stolons, tubers and roots had increasingly higher $^{13}\epsilon$ values. Beside the plant organ, carbon isotope fractionation is influenced by plant age, nematodes and drought. $^{13}\epsilon$ values decreased with plant age in all plant parts, with the exeption of the tubers of the nematode-infected drought-control plants. The leaves and tubers of the nematode-infected plants which were subjected to the late drought period showed increasing $^{13}\epsilon$ values between 70 and 92 days after planting. All plant parts of plants subjected to the early drought period or to potato cyst nematode infection showed higher $^{13}\epsilon$ values than those of the uninfected undroughted control. The late drought period (not shown in Fig. 1) led to similar but weaker responses.

Carbon isotope fractionation determined at each harvest date correlated well with water-use efficiency, from planting until the harvest date, as affected by drought and nematode treatment (Table 1) but not when data from more than one harvest date were pooled. The relation between $^{13}\epsilon$ and water-use efficiency had a high correlation coefficient when the green leaves (r = 0.928) or tubers (r = 0.973) were sampled of the uninfected plants. Nematode infection reduced the correlation (r = 0.580) whereas all treatments of harvest 2 grouped together yielded a correlation coefficient of 0.429. The $^{13}\epsilon$ values of the tubers correlated slightly better with the water-use efficiencies of the whole plant. At the harvest at 70 days after planting, the correlations had worsened because the lines of the early and late drought stressed plants diverged.

Leaves and tubers (shown in Fig. 2, but the same applies to the other plant parts) of infected plants had higher $^{13}\epsilon$ values but infected plants showed lower water-use

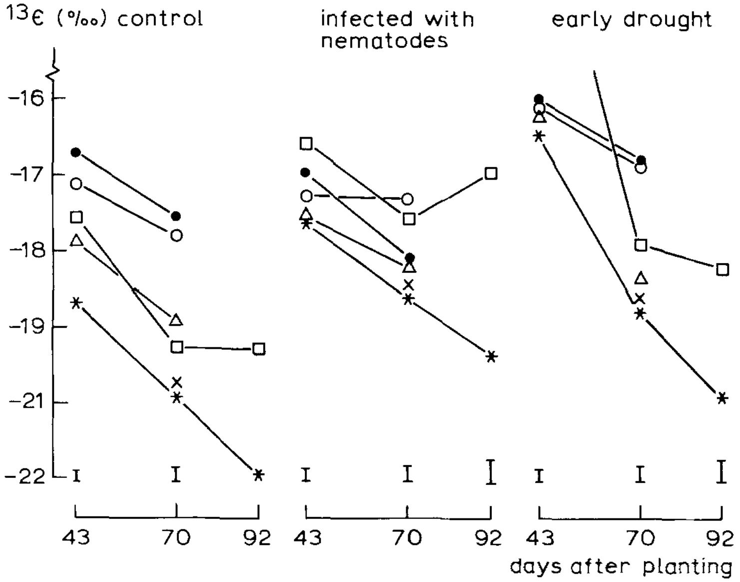

Fig. 1. $^{13}\epsilon$ values of the green (∗) and yellow (×) leaves, stems (△), tubers (□), stolons (○) and roots (●) at 43, 70 and 92 days after planting of the control, the undroughted nematode infected, and the uninfected early droughted plants. Vertical bars represent the $LSD_{0.05}$ values.

efficiencies than the control at the first harvest. At the second and third harvest the nematode infected plants still had higher $^{13}\epsilon$ values but also higher water-use efficiencies than the control.

Table 1. Regression analysis of water-use efficiency (W) versus $^{13}\epsilon$ value (E): $E = S \times W + I$, treatment codes n1 = uninfected, n2 = infected, d1 = drought control, d2 = early drought, d2 = late drought (d.a.p. = days after planting).

Harvest (d.a.p.)	Plant part	Number observations	Combination of treatments	Slope (S)	Intercept (I)	Correlation (r)
43	green leaves	6	n1, d1, d2	1.07	−27.5	−0.928
		6	n2, d1, d2	0.49	−21.5	−0.580
		12	n1, n2, d1, d2	0.35	−21.0	−0.429
	tubers	6	n1, d1, d2	2.41	−36.3	−0.973
		6	n2, d1, d2	1.19	−24.5	−0.612
		12	n1, n2, d1, d2	1.15	−25.0	−0.662
70	green leaves	6	n1, d1, d2, d3	0.66	−26.2	−0.480
		6	n2, d1, d2, d3	0.51	−24.2	−0.658
		12	n1, n2, d1, d2, d3	0.67	−25.9	−0.489
	tubers	6	n1, d1, d2, d3 1.16	−29.7	−0.684	
		6	n2, d1, d2, d3	0.80	−25.3	−0.803
		12	n1, n2, d1, d2, d3	1.12	−28.8	−0.540

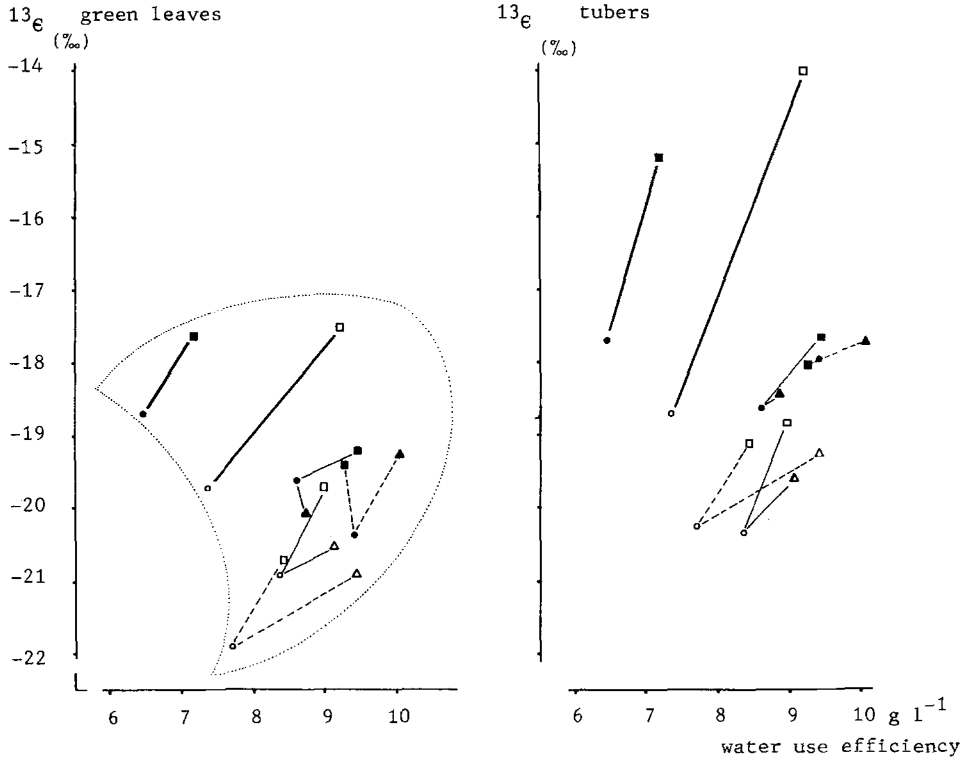

Fig. 2. $^{13}\epsilon$ values of the green leaves and the tubers versus water-use efficiency. Open symbols: uninfected, closed symbols: infected with potato cyst nematodes. Circles: drought control; squares: early drought period, triangles: late drought period. Thick drawn lines connect points of the first, thin drawn lines of the second and broken lines connect points of the third harvest. The dotted line encompasses the range of all values observed (see also Fig. 4).

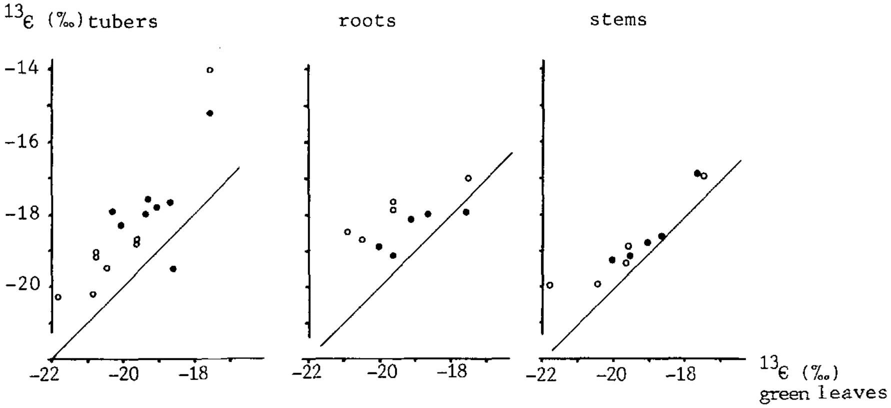

Fig. 3. Relationship between $^{13}\epsilon$ values of the green leaves and the tubers, roots and stems of the three harvests. Open circles: uninfected plants; closed circles: infected plants. Roots and stems observed at the first two harvests only.

$^{13}\epsilon$ values of the various plant parts correlated well (Fig. 3), especially those of the green leaves and the stems. Almost all $^{13}\epsilon$ values of the lower plant parts were higher than those of plants parts that are situated higher up in the plant. With the $^{13}\epsilon$ values of the green leaves on the abscissae, most of the points in Fig. 3 are above the $y = x$ line. Relative to the green leaves, nematode infection decreased the $^{13}\epsilon$ values in the roots, but nematodes did not influence the relation in the other plant parts.

Discussion

Farquhar et al (1982) demonstrated that carbon isotope fractionation is mainly determined by the ratio of intercellular and ambient CO_2 concentration (C_i/C_a). This ratio decreases when C_i decreases as a result of partial stomatal closure during drought. The ratio increases when the photosynthetic rate decreases, for instance due to to a reduced light intensity. As a consequence, discrimination against ^{13}C decreases (higher $^{13}\epsilon$ values), during drought with increased water-use efficiencies. When the photosynthetic rate decreases, ^{13}C discrimination increases and the water-use efficiency decreases. The general trends of the data presented in the Figs. 1 and 2 are schematically represented in Table 2.

Plant age. In all plant parts, $^{13}\epsilon$ values decreased with the age of the plant indicative of an increase of ^{13}C discrimination. This was not associated with a lower but with a higher water-use efficiency. Respired CO_2 was found to be 8 ‰ richer in ^{13}C than the plant (Farquhar et al., 1982). Whence discrimination increases with the age of the plant.

The water-use efficiency of senescing plants declined showing that photosynthesis lagged behind transpiration. This was likely due to nutrient depletion leading to a reduction of photosynthesis. There was no systematic change over time in the vapour pressure deficit between the harvest dates. As the air was not systematically drier later in the season than earlier on, changing humidity of the air could not have contributed to the age effect on water-use efficiency. Tanner and Sinclair (1983) observed a positive correlation between water-use efficiency and the availability of N, P and K nutrients until yields were half that of well fertilized soils. A reduced photosynthesis leads to

Table 2. Trends in $^{13}\epsilon$ values ($^{13}\epsilon$), ^{13}C discrimination, (Discr.), effects on the intercellular CO_2 concentration inferred from Farquhar et al. (1982) (C_i) and water-use efficiency (WUE) (In italics: plant responses observed by Farquhar et al. 1982).

Treatment	$^{13}\epsilon$	Discr.	C_i	WUE	Remarks
Drought	>	<	<	>	e.g. Vos and Groenwold (1989)
Older plants	<	>		>	Respiration increases discrimination
Senescing plants	<	>	>	<	WUE reacts as in infected young plants
Infected young pl.	>	<	<	<	inverse reaction of old plants
Infected old. pl.	>	<	<	>	react like water stressed plants
Higher plant parts	<	>			leaves respire more, hence discriminate more
Less light	<	>	>	<	*nearing compensation point*
Phot. impaired	<	>	>	<	*e.g. in senescing plants*

increased intercellular CO_2 concentrations, increased ^{13}C discrimination and to lower $^{13}\epsilon$ values. These plant responses are similar to those when photosynthesis is reduced due to a reduction of the light intensity. Smedley et al. (1991) found a decrease of ^{13}C discrimination in perennial grasses in the course of the growing season, but this was due to the increasing drought to which the plants were subjected. A possible seasonal change in vapour deficit was not accounted for by Smedley et al. (1991).

Drought. Dry soil conditions cause water stress in plants leading to decreased leaf water potentials, increased stomatal resistance and a more efficient use of water (Haverkort et al. 1991). Table 2 shows increased $^{13}\epsilon$ values due to decreased intercellular CO_2 concentrations, leading to a reduced discrimination of ^{13}C in the leaves and other plant parts. These data corroborate those of Farquhar and Richards (1984) with winter wheat and of Vos and Groenwold (1989) with potato. At the second harvest the plants had been submitted to an early as well as a late drought period. The late drought period had the strongest effect on the water-use efficiency. At similar water-use efficiencies, the late drought stressed plants showed lower $^{13}\epsilon$ values. This is probably because the early water stressed plants, like the nematode infected plants, initially grew slower due to a slowed down plant metabolism which led to a reduced extrusion of ^{13}C. Due to an earlier reduced growth they will have depleted soil nutrients to a lesser extent.

Nematode infection. Infected young potato plants, like water stressed plants, showed higher $^{13}\epsilon$ values but contrary to water stressed plants, they showed reduced water-use efficiencies (Fig. 1). Haverkort et al. (1991) concluded that reduced photosynthesis was likely to be partly responsible for reduced growth and low water-use efficiencies. That $^{13}\epsilon$ values were increased by nematode infection might indicate that the effect of reduced metabolism on $^{13}\epsilon$ values was greater than the reduced photosynthesis effect on $^{13}\epsilon$ values. When the plants were maturing, nematode infection showed similar responses as drought with regard to ^{13}C fractionation and water-use efficiency.

The direct effect of invading juveniles on photosynthesis appearently had disappeared at 43 days after harvest. The most important reason for the increased $^{13}\epsilon$ values of young nematode infected plants, however, was that these plants grew slower, and, probably had extruded less ^{13}C than the control plants. Younger plants which had respired for a shorter period, had higher $^{13}\epsilon$ values as is shown in Fig. 1.

Plant part. Green leaves had the lowest $^{13}\epsilon$ values whereas the roots (harvest at 43 days after planting) or the tubers (at 70 and 92 days) had the highest $^{13}\epsilon$ values of all plant parts (Fig. 1) This was likely due to the higher respiration rates in the leaves than in the other plant parts. Respired CO_2 was shown to be richer in $^{13}CO_2$ in winter wheat than the plant (Farquhar et al. 1982). Tubers, once the photosynthates are incorporated in the starch grains, have low respiration rates hence they show less discrimination of ^{13}C and thus show higher $^{13}\epsilon$ values. The roots of nematode infected plants had lower $^{13}\epsilon$ values than those of the control plants. This may have been caused by the reduction of new root growth because of the infection. The roots of control plants on average were younger than those of the infected plants and, as was discussed before, younger plants have higher $^{13}\epsilon$ values.

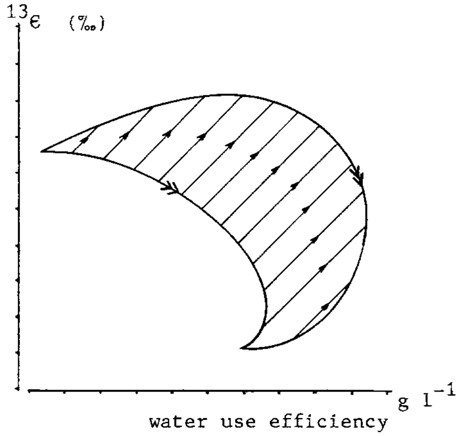

Fig. 4. Range of $^{13}\epsilon$ and water-use efficiency values expected in the course of growth of any crop. Single arrows indicate the influence of plant water stress, double arrows show the response of increased plant age.

Conclusions. In general a good agreement was found between water-use efficiency and carbon isotope fractionation of plants of the same age and with the same health status with regard to nematode infection. Fig. 4 schematically represents the range of $^{13}\epsilon$ versus water-use efficiency relationships found in the experiment. The arrows show the linear relationships between $^{13}\epsilon$ of a certain plant part and water-use efficiency on a certain date. Nematode infection (which initially reduces ^{13}C discrimination because they grow slower for reasons discussed by Fasan & Haverkort (1991) and by Haverkort et al. (1991) related to depletion of soil nutrients) moves the lines to the left, with age the lines move to the right. This research leads to the conclusion that a major part of the variation of the correlation between water-use efficiency and the carbon isotope effect (beside the reasons given by Hubick et al. 1986), is explained by factors which influence the age and the growth rate of the plant.

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