Plant and Soil **154**: 81–88, 1993.

PLSO IAOPN-092

Effects of fertilization levels in two farming systems on senescence and nutrient contents in potato leaves

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Key words: farming system, fertilization, magnesium, nitrogen, potassium, potato, proteolytic enzymes, senescence, *Solanum tuberosum* L.

Abstract

The influence of fertilization on senescence and nutrient remobilization in potato leaves was investigated in two farming systems on a soil with a poor potassium availability. The 'Conventional' farming system followed good local practices including industrial products, while in the 'Bio-Dynamic' farming system industrial fertilizers and synthetic pesticides were avoided. Potassium concentrations in the dry matter of mature leaves varied over a wide range. Nitrogen compounds (protein, chlorophyll) were less affected, and phosphorus concentrations in the dry matter were similar. Magnesium and potassium concentrations in the leaves were negatively correlated. In both farming systems senescence was advanced in plants with a low nutrient supply. Alkaline pyrophosphatase and aminopeptidase activities (in general highest in expanding and mature leaves) were lower and endopeptidase activities peaked earlier on plots with low fertilizer levels. A high percentage of potassium was remobilized from senescing leaves on unfertilized plots, but the phosphorus concentration remained high at the end of the season. The results suggest that the differential net remobilization of nitrogen, phosphorus and potassium depended on actual source/sink relations in the plants.

Introduction

Leaf senescence leads to the death of the organ and is characterized by a net degradation of proteins and of chlorophyll as well as by changes in the enzyme pattern (Noodén and Leopold, 1988). Mobile nutrients can be remobilized from senescing leaves and translocated via the phloem to other plant parts (Hanway and Weber, 1971; Marschner, 1986; Schenk and Feller, 1990). Expanding leaves and tubers may act as major sinks in potato plants. In mature leaves nitrogen is mainly present in chloroplast constituents (e.g. proteins), which must be degraded prior to the export of the nitrogen during senescence (Feller, 1990). Proteins can be hydrolyzed by a series of exo- and endopeptidases to free amino acids (Dalling, 1986; Ryan and Walker-Simmons, 1981). The activities of various peptide hydrolases change differently during the senescence of corn (Feller et al., 1977) and wheat (Feller, 1983; Fröhlich and Feller, 1992) leaves. Potato tubers and leaves are rich in proteinase inhibitors (Ryan 1973; Santarius and Belitz, 1978). However, these inhibitors do not affect major endogenous proteinases and it is therefore unlikely that they are involved in the control of intracellular proteolysis (Santarius and Belitz, 1978). The enzyme pattern changes during senescence in favour of catabolic enzymes (Feller, 1990; Fröhlich and Feller 1991; 1992). Although several aspects of senescence have been investigated in detail, only very restricted information concerning the effects of the nutrient status on leaf senescence, on the change in the enzyme pattern and on the nutrient redistribution in the field is available (Noodén and Leopold, 1988). Recently it was reported that the phosphorus nutrition does not play a major role in the control of leaf senescence in soybean (Crafts-Brandner, 1992).

A large field study (DOC) with three farming systems (Bio-*D*ynamic, *O*rganic and *C*onventional) was started in 1978 on a loamy loess soil in Therwil near Basel (Besson and Niggli, 1991). Potato cultivation followed after a two year period with a grass/clover mixture. Details concerning fertilization, plant protection, yield and some quality parameters for potato tubers were published previously (Besson et al., 1991). This field study offered the unique chance to investigate the influence of the nutrient supply on senescence and nutrient remobilization. The aim of the work presented here was to identify in two farming systems the effects of fertilization on the nutrient dynamics in potato plants by analyzing macronutrient contents and selected enzyme activities in a particular leaf throughout the growing season.

Materials and methods

The experimental design of the 'DOC' field study on Birsmattenhof in Therwil near Basel was reported previously (Besson and Niggli, 1991). Plots with the different treatments considered for the work presented here were available in 4 replicates on a loamy loess soil. Plants grown on fertilizer level 0 (unfertilized), on fertilizer level 1 and on fertilizer level 2 (2-fold quantity applied on level 1) were analyzed for the farming systems "Bio-Dynamic" (D) and "Conventional" (C). The nutrient supply by fertilization in these two farming systems is listed in Table 1 for the growth seasons investigated. Potatoes (*Solanum tuberosum* L., cv. Ostara) were grown in 1981 and 1982 on different plots following a two year period with a grass/clover mixture (Besson and Niggli, 1991; Besson et al., 1991). The tuber yield as well as the availability of K and P in the soil before planting and after harvest are summarized in Table 2. The contents of mineral nitrogen in the soil were far less affected by fertilization than the contents of K and P (Alföldi et al., 1992). Leaf samples (each containing 6 well developed peripheral leaflets) were collected repeatedly during the growing season and transported on ice to the laboratory. At the first sampling date these leaflets were fully expanded at the top of the plant. To detect physiological changes in a particular leaf (time courses), leaflets from the same position were collected throughout the season. Samples for the analysis of enzyme activities and of nitrogen compounds were stored frozen (−20°C) prior to the extraction. Samples for the quantification of K, Mg, Ca and P were dried at 100°C for 24 h. The dry matter was weighed and all results were computed on a dry matter basis.

Samples containing 6 frozen leaflets were extracted in 24 mL extraction buffer (50 m*M* Na-acetate pH 5.4, 0.1% mercaptoethanol, and 1% polyvinylpolypyrrolidone) as described previously (Feller, 1983). The homogenate was filtered through Miracloth (Calbiochem, San Diego) and the filtrate was used to analyze chlorophyll

Table 1. Nutrient supply by fertilization in the farming systems 'Bio-Dynamic' (D0, D1, D2) and 'Conventional' (C0, C1, C2). The values for D1 and C1 were calculated from Besson et al. (1991). Other plots in the same farming systems were fertilized with 200% of the nutrient quantities listed (D2, C2) or remained unfertilized (D0, C0)

Treatment	Fertilization (kg ha^{-1})				
	N	P	K	Ca	Mg
1981					
D1	51	16	17	129	13
C1	127	39	184	59	23
1982					
D1	84	26	46	134	16
C1	123	33	142	38	14

Table 2. Yield (fresh weight of tubers) and nutrient availability in the soil (calculated from Besson et al., 1991, 1992). Nutrients in the soil were analyzed before fertilization and planting as well as after harvest in the farming systems 'Bio-Dynamic' (D0, D1, D2) and 'Conventional' (C0, C1, C2). Unfertilized plots (D0, C0) were compared with those on low (D1, C1) and higher (D2, C2) fertilizer levels

Measurement	Farming system and fertilization level					
	D0	D1	D2	C0	C1	C2
1981						
Yield (t ha^{-1})	15.4	20.6	29.1	22.2	49.1	57.9
K before planting (mg kg^{-1})	6.6	5.8	5.0	5.0	5.8	8.3
K after harvest (mg kg^{-1})	5.0	5.2	6.1	4.2	6.1	15.4
P before planting (mg kg^{-1})	1.2	1.3	1.4	1.7	2.0	2.4
P after harvest (mg kg^{-1})	1.1	1.3	1.6	1.0	2.0	2.8
1982						
Yield (t ha^{-1})	20.5	33.6	43.1	21.1	54.8	62.4
K before planting (mg kg^{-1})	5.0	5.0	5.0	4.2	5.0	8.3
K after harvest (mg kg^{-1})	4.2	3.7	5.0	3.7	6.5	12.3
P before planting (mg kg^{-1})	0.8	0.7	0.8	0.8	0.7	1.7
P after harvest (mg kg^{-1})	0.7	0.9	1.3	0.7	1.3	2.0

(Strain et al., 1971), total proteins (Bradford, 1976) and free amino groups with a ninhydrin reagent (Cramer, 1958). A part of the extract was centrifuged for 10 min at 4000 *g* and the supernatant was desalted by centrifugation through Sephadex G-25 as described by Feller et al. (1977). The desalted extract was used to measure the activities of : aminopeptidase, with L-leucine-p-nitroanilide as substrate; carboxypeptidase, with N-carbobenzoxy-L-phenylalanine-L-alanine as substrate; endopeptidase at pH 5.4 with azocasein as substrate (Feller, 1983); and pyrophosphatase (Salgo and Feller, 1986).

Samples of 100 mg dry matter were kept in glass tubes for 4.5 h at 540°C. The ash was dissolved in 0.5 mL 10 *N* HCl and 49.5 mL H_2O were added. This solution was used to determine P colorimetrically with a vanadate-molybdate reagent. The elements K, Mg and Ca were detected by atomic absorption spectrometry after appropriate dilution with 1.267 gL^{-1} CsCl suprapur in 0.1 *N* HCl (for K) or with 13.37 gL^{-1} $LaCl_3$ 7 H_2O in 0.1 *N* HCl (for Mg and Ca).

Results

From the study in 1981 (fourth year after the change to different farming and fertilization procedures) it became evident that senescence and nutrient remobilization in potato leaves strongly depended on the nutrient supply. Senescence, as judged by the net chlorophyll degradation, was advanced on unfertilized plots compared to the other treatments (Table 3). Potassium concentrations in the dry matter of mature leaves varied over a very wide range and were highest on C2 plots. Magnesium and calcium concentrations were far less influenced. Pyrophosphatase activity (an enzyme involved in biosynthetic processes) was lower on unfertilized plots. Azocaseinase (a catabolic enzyme) reached highest activities on well fertilized plots later than on plots with a low nutrient supply.

The investigation was repeated in 1982 (fifth season in the field study) on other plots subjected to the same farming and fertilization techniques. The main results of the two seasons were consistent (Table 3; Figs. 1, 2 and 3). The concentrations of some macronutrients are shown in Figure 1 and indicate that potassium was most severely affected by the fertilization level. In general, magnesium concentrations in the leaves were highest on unfertilized plots. On the other hand calcium concentrations in the dry matter were far less influenced and increased only slightly throughout the growing season. Phosphorus levels in the leaves were very constant. The highest percentage of the potassium present in mature leaves was remobilized on

Table 3. Senescence and macronutrients in leaves of potato plants grown during summer 1981 in the farming systems 'Bio-Dynamic' (D0, D1, D2) and 'Conventional' (C0, C1, C2). Unfertilized plots (D0, C0) were compared with those on low (D1, C1) and higher (D2, C2) fertilizer levels. Means of 4 replicates are listed. Values in the same row followed by the same letter are not significantly different at the p 0.05 level (Student-Newman-Keuls test)

Date	Farming system and fertilization level					
	D0	D1	D2	C0	C1	C2
Potassium (mg K g^{-1} dry matter)						
June 2	10.7 c	13.1 c	20.0 bc	17.1 c	27.9 ab	31.8 a
June 30	13.0 d	14.2 d	22.0 c	13.6 d	34.7 b	45.5 a
July 28	5.7 c	8.6 c	13.2 c	8.9 c	26.8 b	46.9 a
Magnesium (mg Mg g^{-1} dry matter)						
June 2	3.1 a	3.5 a	3.5 a	4.4 a	2.8 ab	2.0 b
June 30	10.6 ab	10.0 ab	8.8 a	10.6 ab	5.9 b	3.5 c
July 28	12.4 ab	13.5 ab	12.3 a	14.5 a	6.6 b	3.5 c
Calcium (mg Ca g^{-1} dry matter)						
June 2	14.1 a	17.3 a	16.7 a	15.4 a	14.5 a	14.2 a
June 30	36.5 ab	38.4 ab	39.4 a	32.3 ab	31.1 b	23.4 c
July 28	36.9 ab	38.3 ab	40.5 a	37.5 ab	32.6 ab	28.3 b
Chlorophyll (mg chlorophyll g^{-1} dry matter)						
June 2	3.4 b	3.5 b	3.7 b	3.7 b	4.4 a	4.6 a
June 30	3.1 b	3.1 b	3.3 b	3.3 b	4.7 a	5.4 a
July 28	0.7 c	1.0 c	1.5 bc	1.2 bc	2.0 ab	2.6 a
Pyrophosphatase activity (mmol h^{-1} g^{-1} dry matter)						
June 2	5.4 d	5.5 d	5.8 d	6.6 c	7.9 b	8.6 a
June 30	3.0 a	3.0 a	3.0 a	3.0 a	3.8 a	4.4 a
July 28	0.1 a	0.3 a	0.6 a	0.4 a	0.7 a	0.9 a
Azocaseinase activity (mg h^{-1} g^{-1} dry matter)						
June 2	5.2 a	5.6 a	5.6 a	5.3 a	4.6 a	3.5 a
June 30	21.8 a	20.3 a	21.5 a	18.7 a	20.9 a	19.1 a
July 28	10.2 c	13.6 c	15.2 bc	15.2 bc	20.2 ab	21.7 a

unfertilized plots. In contrast, major quantities of phosphorus were exported only from the leaves on well fertilized plots.

The dry matter per leaflet was only slightly increased on plots with a high nutrient supply (Fig. 2). Protein and chlorophyll levels in mature leaves increased by 20 to 50% from unfertilized (D0, C0) to well fertilized (C2) plots. The net decrease in the chlorophyll and protein quantities was more pronounced in plants with a low nutrient supply. No accumulation of free amino acids was observed during the net degradation of proteins.

Pyrophosphatase activity was initially (June 8) very similar for all treatments, but it decreased more rapidly on unfertilized plots (Fig. 3). Similar tendencies were observed for leucine aminopeptidase. No clear trend was detected for carboxypeptidase. The activity of this vacuolar enzyme remained high during the period of net protein degradation. Azocaseinase activity (endopeptidase) showed interesting time courses for the various treatments. At the end of the season this enzyme was more active in plants on well fertilized plots.

Discussion

The fertilization effects were more pronounced on the 'Conventional' than on the 'Bio-Dynamic' plots. However, it must be borne in mind that at the same fertilization level more macronutrients were applied to 'Conventional' plots (Besson et

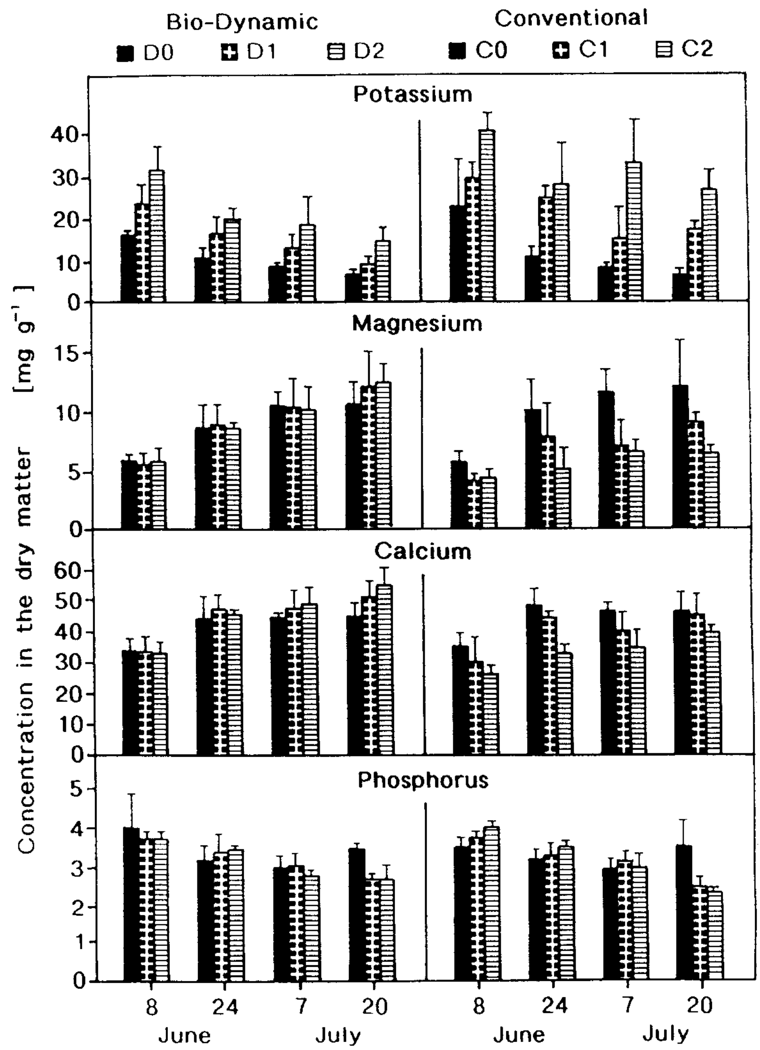

Fig. 1. Concentrations of potassium, magnesium, calcium and phosphorus in the dry matter of potato leaves collected during summer 1982. Unfertilized plots (D0, C0) were compared with those on low (D1, C1) and higher (D2, C2) fertilizer levels in the farming systems 'Bio-Dynamic' and 'Conventional'. Means and standard deviations of 4 replicates are shown.

al., 1991). Taking this fact into consideration, the influence of the fertilizer quantity on the parameters investigated in this study were similar for the two farming systems. Although phosphorus, potassium and magnesium are mobile within the plant (Marschner, 1986; Schenk and Feller, 1990), a marked loss from senescing leaves was observed only for potassium. Our results lead to the conclusion that potassium was limiting on unfertilized plots. The concentration of this element in the dry matter of leaves varied over a very wide range. Considerable potassium quantities were lost from senescing leaves on plots with a low potassium supply. Nitrogen compounds (chlorophylls, proteins) decreased later than potassium. No major effects of the growth conditions on the level of free amino acids was observed. This result indicates that the metabolism and the translocation of amino acids via the phloem were rapid enough to avoid an accumulation. The increased magnesium concentrations in leaves with a low potassium level were most likely caused by a compensation during the nutrient uptake into the roots. A more rapid leaf senescence was detected in unfertilized plants. The activities of the investigated enzymes were

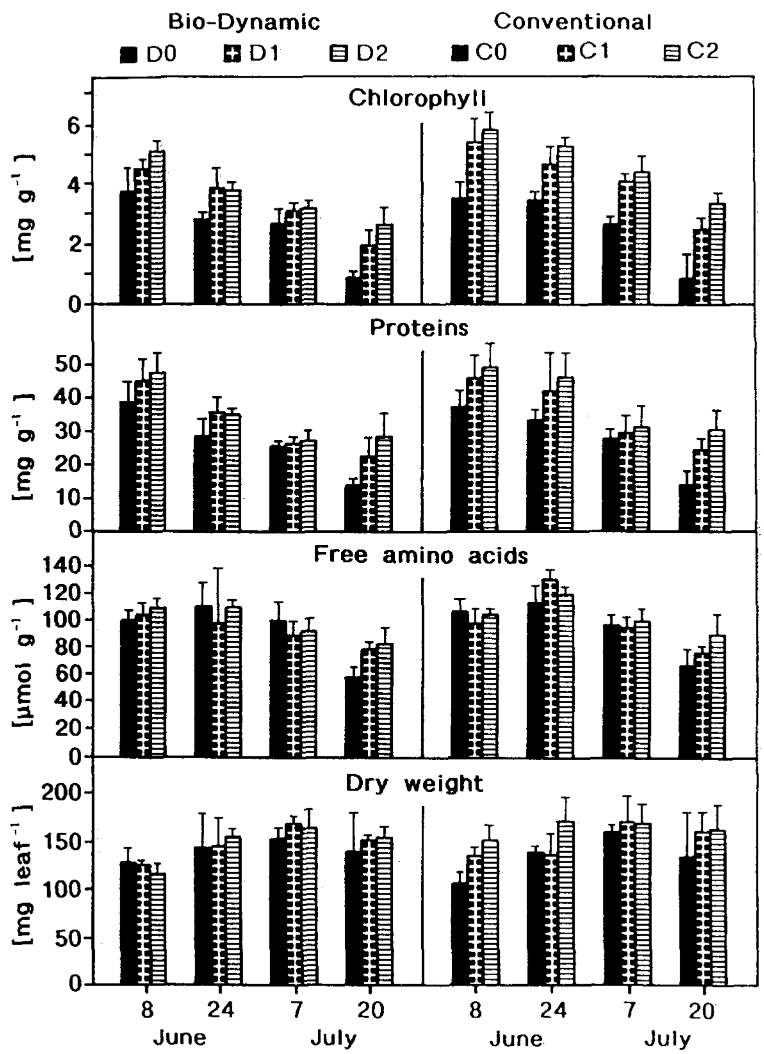

Fig. 2. Nitrogen remobilization in potato leaves during summer 1982. Unfertilized plots (D0, C0) were compared with those on low (D1, C1) and higher (D2, C2) fertilizer levels in the farming systems 'Bio-Dynamic' and 'Conventional'. Means and standard deviations of 4 replicates are shown.

similar on a dry matter basis for all treatments in young leaves, but the changes in the enzyme pattern from anabolic (pyrophosphatase) to catabolic (endopeptidase) activities occurred earlier in plants with a low nutrient supply. Aminopeptidase was most active in young and mature leaves compared to senescing leaves. The changes in the enzyme pattern from anabolic to catabolic activities were clearly influenced by the availability of nutrients in the soil. The properties of young leaves were less affected by fertilization than the nutrient losses from senescing leaves.

Acknowledgements

We thank Dr A Fleming for improving the English of the manuscript. Financial support for the DOC field study was provided by the Swiss Federal Office for Agriculture. The senescence

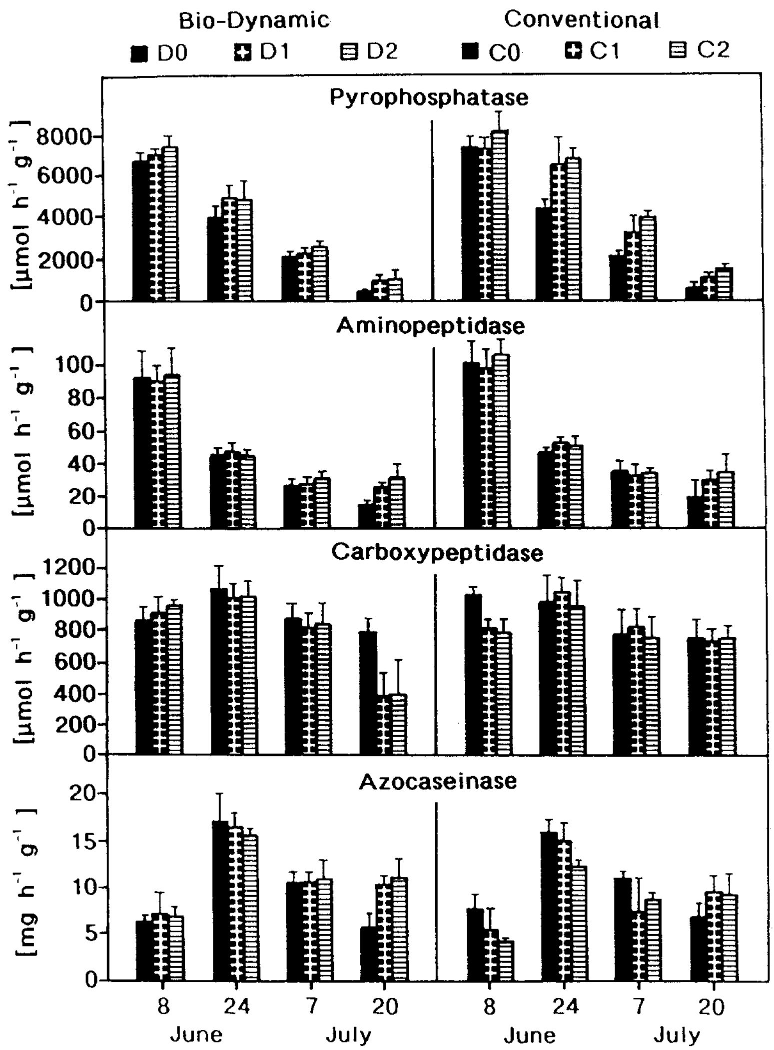

Fig. 3. Activities of pyrophosphatase and of peptide hydrolases in potato leaves collected during summer 1982. Unfertilized plots (D0, C0) were compared with those on low (D1, C1) and higher (D2, C2) fertilizer levels in the farming systems 'Bio-Dynamic' and 'Conventional'. Means and standard deviations of 4 replicates are shown.

investigations were partially supported by Swiss National Science Foundation (Projects 3.067-0.81 and 31-30805.91).

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Section editor: M L van Beusichem