

Potato Performance in Response to Different Mulches. 1. Nitrogen Metabolism and Yield

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This work evaluates the impact that the application of different mulches exerts on nitrogen (N) metabolism in roots and leaves and on yield of potato tubers (*Solanum tuberosum* var. Spunta). The parameters analyzed in this experiment were root temperature; nitrate reductase and nitrite reductase activities; concentrations of nitrates, ammonium, amino acids, proteins, and organic N; dry weight of roots and leaves; and tuber yield. Use of white polyethylene (T2) and white-black plastic (T3) promoted optimal root temperatures for plant growth (23–27 °C). Under these experimental conditions, plants showed the greatest efficiency in N utilization and the greatest yield in tubers. Considering the sensitivity of this plant to thermal stress, high root temperatures caused by black polyethylene (>31 °C) (T4) depressed N metabolism as well as yield, compared with the results of T2 and T3. Finally, clear polyethylene plastic (T1) caused more damage than did the absence of mulch (control, T0).

Keywords: *Solanum tuberosum*; mulches; root temperatures; nitrogen metabolism; yield

INTRODUCTION

Plant growth depends on an adequate supply of nitrogen (N) to form the amino acids, proteins, and nucleic acids necessary for development. In the biosphere, N is available for plants in different forms; this includes molecular N₂, volatile ammonia or NO_x, mineral N, and organic N, and, in well-aerated agricultural soils, mineral N and especially nitrate (NO₃⁻-N) are the most abundant forms of available N (von Wirén et al., 1997).

The first step in NO₃⁻ assimilation, the reduction of NO₃⁻ to NO₂⁻ catalyzed by nitrate reductase (NR), is considered the biochemical rate-limiting step in the N assimilation pathway (Gojon et al., 1998). Considerable attention has been focused on NR, and its activity is highly regulated (Campbell, 1988, 1996). Nitrate availability, growth regulators, light, products of NO₃⁻ assimilation, and other physiological and environmental parameters are all factors in the regulation of NO₃⁻ assimilation (Lillo, 1994; Crawford, 1995; Padgett and Leonard, 1996; Sivasankar and Oaks, 1996; López-Cantarero et al., 1997; Ruiz et al., 1998). Among the environmental parameters, the effect of root temperature was striking for its influence on NO₃⁻ uptake and assimilation (von Wirén et al., 1997).

One technique that has strongly boosted agricultural output in the past few years has been the use of plastic coverings over the root zones of plants. The beneficial effects of mulches on soil moisture, soil and air temperature, soil water availability, soil erosion, root growth,

nutrient availability, and yield, quality, and fruiting response of plants have been studied in a variety of crops (Manrique, 1995; Tilander and Bonzi, 1997; Schmidt and Worthington, 1998). In addition, the effects of mulch color have been evaluated on strawberries (Albregts and Chandler, 1993), tomatoes (Decoteau et al., 1989), and cowpeas (Hunt et al., 1990). These studies have shown that mulch color determines changes in soil temperatures.

Potato plants require relatively large amounts of N for optimal growth and tuber production (Manrique, 1995). An increase or decrease in the root temperature beyond the optimum for good plant growth directly affects N uptake and assimilation (von Wirén et al., 1997). In addition, the potato plant is intrinsically sensitive to heat stress (Manrique, 1995). Considering these factors, we sought to evaluate the effects of different types of mulches on the N metabolism in the roots and leaves of the potato plant, as well as on tuber yield.

MATERIALS AND METHODS

Crop Design. The experiment was conducted for 3 years (1993, 1994, and 1995) in the experimental station of the Centre for Agricultural Research and Training of Granada (Spain). The species used was *Solanum tuberosum* L. var. Spunta, planted at the beginning of March, and the crop cycle was ~4 months. The climate was semiarid and the area intensively used for agriculture. The soil used was arenolimoso with the following characteristics: sand (42.8%), silt (39.7%), and clay (17.5%); CaCO₃ equivalent (9%), active CaCO₃ (2.9%); total N (0.1%); PO₄³⁻ (49.5 ppm); cation-exchange capacity (73.9 mmol kg⁻¹); K⁺ (2.7 mmol kg⁻¹); Na⁺ (4.1 mmol kg⁻¹); pH (H₂O, 7.7); electrical conductivity (EC = 2.76 mΩ⁻¹ cm⁻¹).

The experimental design was a factorial arrangement in a randomized complete block with five treatments. Each treatment was replicated four times, for a total of 20 plots. Each

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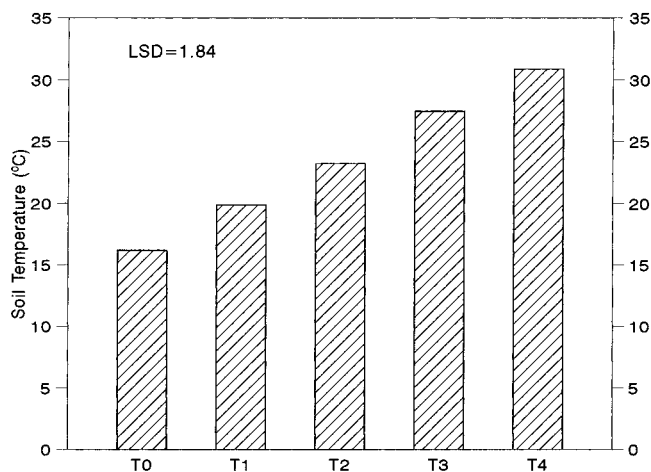


Figure 1. Response of soil temperature to application of different mulches: T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness). All data represent the means for 3 years.

plot had an area of 78.4 m^2 , with a planting density of 4.17 plants m^{-2} . Plants were spaced 30 cm apart, with 80 cm between rows. The irrigation water had the following properties: pH, 7.65; EC = 0.91 mS cm^{-1} ; Cl^- = 58.5 mg L^{-1} ; Na^+ = 25 mg L^{-1} ; K^+ = 4 mg L^{-1} ; H_2CO_3 = 369 mg L^{-1} .

The soil temperature was measured at the 15 cm depth, using probes (107 type) from Campbell Scientific. Root temperature was measured (six measurements at 4 h intervals) every 3 days of the crop cycle. The soil temperatures represented in Figure 1 are the means for each treatment over the duration of the experiment.

The different treatments consisted of covering the entire soil surface of each plot (78.4 m^2) in total with different plastic mulches (polyethylene sheets), making a tight seal with the soil: transparent polyethylene (25 μm in thickness, T1), white polyethylene (25 μm in thickness, T2), coextruded black and white polyethylene (50 μm in thickness, T3), and black polyethylene (25 μm in thickness, T4). Finally, no plastic was applied in the control treatment.

The fertilization used was the same as is habitually applied by farmers in the zone. In the month of February in all three years, N (NH_4NO_3), P (P_2O_5 soluble in water), and K (K_2O soluble in water) were each applied at 27 g m^{-2} . Afterward, and at the end of the month of April, 25 g m^{-2} was applied in the form of NH_4NO_3 . Fertilization was complemented with the following micronutrients: Fe, 0.5 mg L^{-1} ; B, 0.1 mg L^{-1} ; Mn, 0.1 mg L^{-1} ; Zn, 0.075 mg L^{-1} ; Cu, 0.075 mg L^{-1} ; and Mo, 0.05 mg L^{-1} . Iron was applied as FeEDDHA and B as H_3BO_3 , and the remaining micronutrients were applied as sulfates.

Plant Sampling. The plant materials (leaves and roots) were sampled two times every 2 weeks, with the first sampling on May 27 and the second sampling on June 10. These two samplings corresponded to the maturity phase of the plant, the most representative and determinat in the potato crop, given that in this phase the fruits and seeds are produced in addition to the greatest amount of tubers (Manrique, 1995). For each sampling, 10 plants were collected from each replicate per treatment. Leaf samples were taken only from plants with fully expanded leaves of the same size. Leaves were picked at about one-third of the plant height from the plant apex. Roots and leaves were rinsed three times in distilled water after they had been disinfected with nonionic detergent at 1% (Wolf, 1982) and then blotted on filter paper. At each sampling, fresh matter was used for the NR and nitrite reductase (NiR) assays, amino acids, and proteins; a subsample was then dried in a forced air oven at 70 $^\circ\text{C}$ for 24 h, ground in a Wiley mill, and then placed in plastic bags for the further analyses (NO_3^- , NH_4^+ , organic N, dry weight leaf $^{-1}$, and dry weight root $^{-1}$).

Plant Analysis. Detection of *in Vitro* NR and NiR Activity.

At each sampling, portions of roots and leaves were ground in a mortar at 0 $^\circ\text{C}$ with a ratio of 1:10 (w/v) 50 mM KH_2PO_4 buffer (pH 7.5) containing 2 mM EDTA, 1.5% (w/v) soluble casein, 2 mM dithiothreitol (DTT), and 1% (w/v) insoluble polyvinylpyrrolidone. The homogenate was filtered and then centrifuged at 3000g for 5 min, after which the supernatant was centrifuged at 30000g for 20 min. The resulting extract was used to measure enzyme activities NR and NiR. The extraction medium was optimized for the enzymatic activities so that these could be extracted jointly by the same method (Groat and Vance, 1981; Lillo, 1984; Kaiser and Lewis, 1984; Singh and Srivastava, 1986).

The NR assay (EC 1.6.6.1) followed the methodology of Kaiser and Lewis (1984). The 2 mL reaction mixture contained 100 mM buffer KH_2PO_4 (pH 7.5), 100 mM KNO_3 , 10 mM cysteine, 2 mM NADH, and enzyme extract. The NR assay was carried out at 30 $^\circ\text{C}$ for 30 min and stopped by the addition of 1000 mM zinc acetate. The nitrite formed was colorimetrically determined at a wavelength of 540 nm after azocoupling with sulfanilamide and naphthylethylenediamine dihydrochloride (Hageman and Hucklesby, 1971). The NR activity was expressed as micromoles of NO_2^- formed per gram of fresh weight (fw) per hour.

NiR activity (EC 1.7.7.1) was determined by the disappearance of NO_2^- from the reaction medium (Lillo, 1984). The reaction mixture contained 50 mM buffer KH_2PO_4 (pH 7.5), 20 mM KNO_2 , 5 mM methyl viologen, 300 mM NaHCO_3 , and 0.2 mL of enzyme extract. After incubation at 30 $^\circ\text{C}$ for 30 min, the nitrite content was determined and expressed as micromoles of NO_2^- per gram of fw per hour.

Amino Acid and Soluble Protein Determination. Fresh leaf samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH_2PO_4 , pH 7) and centrifuged at 12000g for 15 min. The resulting supernatant was used for the determination of total amino acids by the ninhydrin method as described by Yemm and Cocking (1955); total free amino acids were expressed as milligrams of glycine per gram of fw. Soluble proteins were measured by using Bradford's method (1976), with bovine serum albumin as the standard, and expressed as milligrams of bovine serum albumin per gram of fw.

NO_3^- and NH_4^+ Determinations. NO_3^- -N and NH_4^+ were analyzed from an aqueous extraction of 0.2 g of dried and ground material in 10 mL of Millipore-filtered water; a 100 μL aliquot was taken for NO_3^- -N determination and added to 10% (w/v) salicylic acid in sulfuric acid at 96%, and the NO_3^- -N concentration was measured by spectrophotometry (Cataldo et al., 1975). For the NH_4^+ determination, 100 μL aliquots were taken, performing quantifications as described below, under organic N. The results were expressed as milligrams per gram of dry weight (dw).

Organic N Determination. A 0.1 g sample of dried plant tissue was digested with sulfuric acid with H_2O_2 (Wolf, 1982). After dilution with deionized water, a 1 mL aliquot of the digest was added to the reaction medium containing buffer [5% potassium sodium tartrate, 100 μM Na_2HPO_4 , and 5.4% (w/v) NaOH], 15%:0.3% (w/v) sodium salicylate/sodium nitroprusside and 5.35% (v/v) NaHClO_3 . Samples were incubated at 37 $^\circ\text{C}$ for 15 min, and the organic N was measured (Baethgen and Alley, 1989). The results were expressed as milligrams per gram of dw.

Yield. Plant yield (tubers) was expressed as the mean of tuber weight (kilograms per plant). Collected tubers were weighed on each plant at harvest.

Statistical Analysis. Analysis of variance techniques were used to assess treatment means. ANOVAs were performed on pooled data from three years. Treatment means were compared using the LSD at the 0.05 probability level. Levels of significance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and ns (not significant).

RESULTS AND DISCUSSION

One of the direct effects caused by polyethylene covering the root zone is greater soil temperature, due

to the increase in energy interception and prevention of heat loss from the soil. The overall result was less fluctuation between day and night soil temperatures (Manrique, 1995; Tilander and Bonzi, 1997; Schmidt and Worthington, 1998). Variation in mulch color brings about changes in soil temperature (Decoteau et al., 1989; Hunt et al., 1990; Albrechts and Chandler, 1993; Yunasa et al., 1994). In our experiment, the application of mulches raised the temperature in the root zone in relation to the control ($P < 0.001$; Figure 1). Mean temperatures during the crop cycle under the different types of mulches (Figure 1) showed that the warmest soil resulted from black polyethylene (T4) (31 °C) and the coolest with transparent polyethylene (T1) (19 °C). Black polyethylene absorbs roughly 96% of the short-wave radiation while reflecting very little (Ham et al., 1993). Absorbed radiation conducted to the underlying soil warms the soil (34 °C) (Teasdale and Abdul-Baki, 1997). White polyethylene resulted in cooler soils (26 °C) than did the black (31 °C) because white surfaces reflect most wavelengths of solar radiation (Decoteau et al., 1989; Hatt et al., 1993). Schmidt and Worthington (1998) demonstrated that transparent mulches prevent soil warming, presenting a mean temperature of 18–20 °C during the crop cycle. These works are consistent with our data concerning the effects of different mulches used here (Figure 1).

In the biosphere, plants are exposed to different forms of N, which comprise mineral and organic N forms in the soil, as well as volatile ammonia (NH_3) or nitrogen oxides (NO_x), and molecular N_2 in the atmosphere. The form of N uptake is determined mainly by its abundance and accessibility. Thus, NO_3^- and NH_4^+ are the major N forms for plant nutrition under agricultural conditions. In well-aerated agricultural soils, mineral N (especially NO_3^-) is the form most absorbed and available for plants (von Wirén et al., 1997). Both the NO_3^- level and NR are considered to be limiting factors for growth, development, and production of amino acids and proteins in plants (Campbell, 1996; Sivasankar and Oaks, 1996). One of the factors that determines or influences root absorption of NO_3^- is soil temperature. A reduction in soil temperature (<12 °C) diminishes root uptake of NO_3^- but favors NH_4^+ uptake (Smart and Bloom, 1991; von Wirén et al., 1997). Both the control treatment and the different treatments with polyethylene gave rise to soil temperatures of >12 °C (Figure 1), so no inhibition or decrease in NO_3^- uptake by the root should have occurred.

With respect to NO_3^- values in the roots ($P < 0.001$) and in the leaves ($P < 0.001$) (Table 1), the greatest concentrations were presented with T1, whereas the least were given by T2 and T3. In all treatments, NO_3^- concentrations were greater in the roots than in the leaves (Table 1). The least NO_3^- concentrations were presented by T2 and T3, which were found in the treatments with root temperatures >12 °C (Figure 1), indicating that the behavior of NO_3^- in the roots was possibly independent of the soil temperature caused by the polyethylene, and therefore that the variation in concentrations of this anion was due to the differences in its reduction by NR.

NR catalyzes the reduction of NO_3^- to NO_2^- ; this is considered the biochemical rate-limiting step in the NO_3^- assimilation pathway (Sivasankar and Oaks, 1996; Gojon et al., 1998). Figure 2 shows the NR

Table 1. Accumulation of Various N Forms Due to Application of Different Mulches^a

treatment	mg g ⁻¹ of fw			mg g ⁻¹ of dw		
	amino acid	protein	nitrate	ammonium	organic N	
roots	T0	1.39	9.66	3.52	2.19	15.6
	T1	0.85	7.94	4.34	1.63	13.0
	T2	1.98	11.9	2.93	2.51	17.8
	T3	2.12	12.0	2.79	2.43	17.8
	T4	1.46	9.93	3.61	2.27	15.3
LSD at 0.05	0.03	0.98	0.04	0.03	1.04	
leaves	T0	3.64	19.6	2.51	1.07	12.2
	T1	3.26	17.3	3.37	0.84	9.79
	T2	3.94	21.3	2.01	1.56	14.5
	T3	4.07	21.0	2.06	1.53	14.2
	T4	3.55	19.6	2.67	1.33	12.1
LSD at 0.05	0.67	1.06	0.02	0.02	1.14	

^a All data represent the means of two samplings at plant maturity (first sampling at May 27 and the second sampling at June 10) for 3 years. T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness).

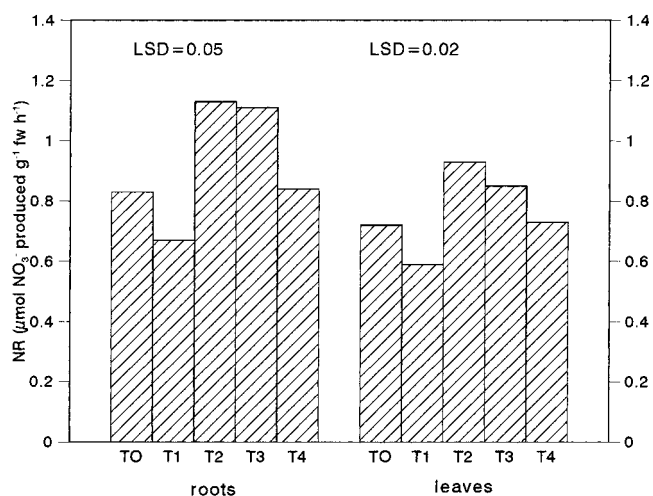


Figure 2. Response of NR activity in roots and leaves to application of different mulches: T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness). All data represent the means of two samplings at plant maturity (first sampling at May 27 and the second sampling at June 10) for 3 years.

activities in the roots ($P < 0.001$) with the leaves ($P < 0.001$). NR behaviors in both organs were similar, given that the least activity values were registered for T1 and the greatest for T2 and T3 (70 and 57%, respectively). The increase in NR with treatments T2 and T3 in both organs implied a greater demand of NO_3^- for their reduction and assimilation. A factor regulating both the increase in NR and its activity is the presence of NO_3^- (Crawford, 1995; Campbell, 1996; Gojon et al., 1998). If treatments T2 and T3 promoted greater NO_3^- input, which stimulated NR activity (Figure 2), this could account for decreased NO_3^- content in these treatments due to its reduction (Table 1, Palauqui et al., 1996; López-Cantarero et al., 1997; Gojon et al., 1998; Ruiz et al., 1998; Ruiz and Romero, 1998). The relationship between both NR and NO_3^- in the roots and in the leaves was negative (roots, $r = -0.93^{***}$; leaves, $r = -0.95^{***}$).

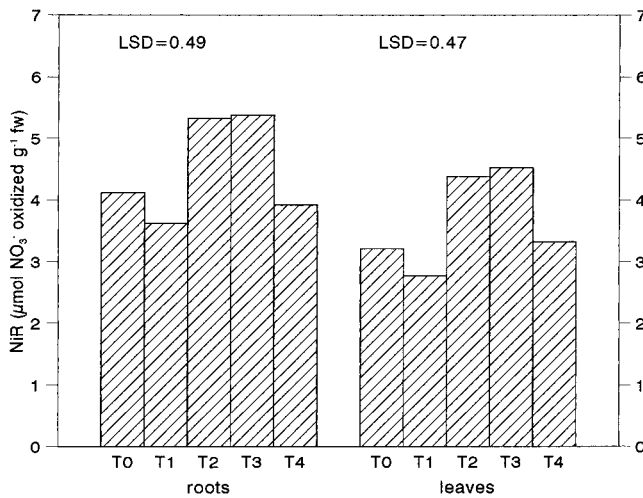


Figure 3. Response of NiR activity in roots and leaves to application of different mulches: T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness). All data represent the means of two samplings at plant maturity (first sampling at May 27 and the second sampling at June 10) for 3 years.

The next step in NO_3^- assimilation is the conversion of NO_2^- to NH_4^+ by the action of NiR (Sivasankar and Oaks, 1996). Both enzymes, NR and NiR, are induced by the same factors (Oaks, 1994), accounting for their similar responses to mulch treatments (Figure 3). The greatest enzymatic activities were presented at T2 and T3, whereas the least were at T1, both in the roots ($P < 0.001$) and in the leaves ($P < 0.001$). Regardless of the mulch types used and because NO_2^- is toxic, the greater NiR activity, relative to NR activity, was expected (Pécsváradí and Zsoldos, 1996).

The NH_4^+ values in our experiment resulted from the reduction of NO_3^- and of the N source applied as fertilizer. The greatest NH_4^+ concentrations in both organs were presented at T2 and T3 ($P < 0.001$; Table 1). These results are logical if it is taken into account that the treatments T2 and T3 caused perhaps the most intense reductions of NO_3^- , given the greatest NR activities (Figure 2). Again, the greatest NH_4^+ levels, regardless of the treatment applied, were recorded in the roots (Table 1). Therefore, we conclude that the reduction and assimilation of NO_3^- as well as the incorporation of NH_4^+ to organic molecules occurred in roots.

The greatest concentrations of amino acids and proteins, both in the roots ($P < 0.001$) and in the leaves ($P < 0.01$), were presented by treatments T2 and T3 (Table 1), whereas the least were given by T1. The relationships in our experiment between the NR activity and these N compounds were positive (roots, NR activity–amino acids, $r = 0.95^{***}$; NR activity–proteins, $r = 0.91^{***}$; leaves, NR activity–amino acids, $r = 0.77^{**}$; NR activity–proteins, $r = 0.87^{***}$). Organic N is another parameter that strengthened with intensified NO_3^- reduction (Vincentz et al., 1993). As in the case of the amino acids and proteins, the organic N (Table 1) concentration correlated with the increases in NR activity (roots, $P < 0.001$; leaves, $P < 0.001$), because the greatest concentrations were found at T2 and T3 and the least at T1. The relationship between the NR activity and the organic N concentration was positive (roots, $r = 0.84^{***}$; leaves, $r = 0.92^{***}$). The levels of

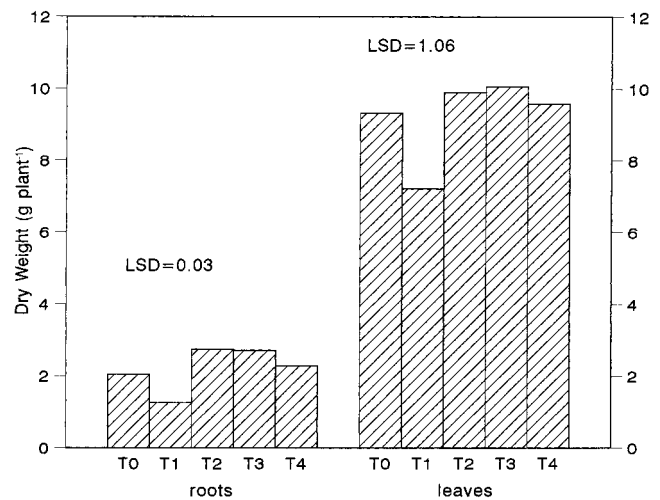


Figure 4. Response of dry weight of roots and leaves to application of different mulches: T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness). All data represent the means of two samplings at plant maturity (first sampling at May 27 and the second sampling at June 10) for 3 years.

the amino acids and proteins were greater than in the leaves, whereas organic N reached its greatest concentrations in the roots, possibly because of a greater incorporation of amino acids and proteins to nitrogenous organic compounds (Table 1).

We found that treatments T2 and T3 most intensified the processes of N metabolism. Root temperatures resulting from the application of white and white-and-black polyethylene promoted the greatest absorption of NO_3^- and NH_4^+ , greater incorporation of these mineral forms into organic compounds, the greatest activities of NR and NiR, and the greatest concentrations of amino acids, proteins, and organic N. Therefore, we can define the root temperatures of T2 and T3 as optimal in relation to the N metabolism for cultivating the potato. Finally, the increased root temperature promoted by T4 (black polyethylene) appears to reduce the processes related to N metabolism. Root temperature outside the optimal range for good growth of a given plant directly affects N uptake and assimilation (von Wirén et al., 1997). In addition, it must be taken into account that the potato is intrinsically sensitive to heat stress (Manrique, 1995).

Nitrogen metabolism plays an important role in the growth and development of most plants (Silberbush and Lips, 1991). The greatest levels of the dry weight of roots ($P < 0.001$) and leaves ($P < 0.001$; Figure 4) were recorded for T2 and T3, whereas the least were given by T1, representing decreases of 54 and 28%, respectively. The relationship of the different N forms and the dry weight was positive (roots, protein–dry weight, $r = 0.87^{***}$; amino acids–dry weight, $r = 0.97^{***}$; organic N–dry weight, $r = 0.88^{***}$; leaves, proteins–dry weight, $r = 0.80^{**}$; amino acid–dry weight, $r = 0.72^{**}$; organic N–dry weight, $r = 0.79^{**}$), reflecting the close relationship between N metabolism and biomass production (Mattson et al., 1991; Ruiz et al., 1998).

The yield behavior was similar to that of the dry weight, given that the greatest yields resulted with T2 and T3, whereas the least resulted with T1 ($P < 0.001$; Figure 5). These results confirm the relationship between the production and efficiency in N metabolism

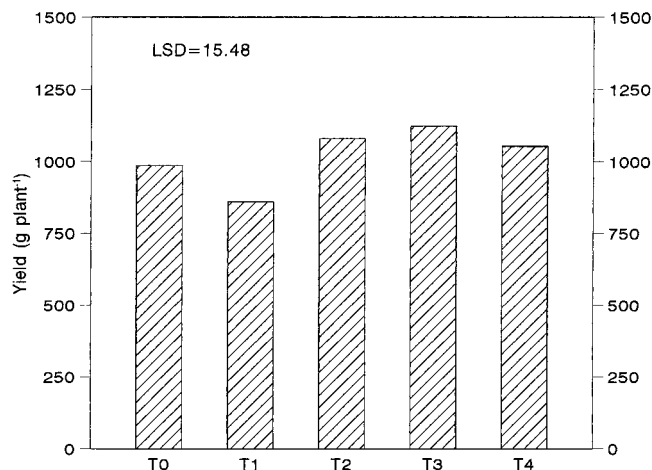


Figure 5. Response of tuber yield to application of different mulches: T0, no plastic, control treatment; T1, transparent polyethylene (25 μm in thickness); T2, white polyethylene (25 μm in thickness); T3, coextruded black and white polyethylene (50 μm in thickness); T4, black polyethylene (25 μm in thickness). All data represent the means for 3 years.

and use promoted by the T2 and T3 treatments (Manrique, 1995). Similar results have been reported in other plants under different growing conditions (López-Cantarero et al., 1997; Ruiz and Romero, 1998).

Finally, the growth and yield obtained in potato plants cultivated with the transparent polyethylene coincide with a decline in the synthesis of photoassimilates (data not shown). Taking into account the essential role of the carbohydrates as an energy source in N metabolism (Lawlor et al., 1995), as well as in the formation and production of tubers in potato plants (Manrique, 1995), could explain the poor efficiency of N utilization and poor yield obtained with the treatment T1.

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