leaves, and thrips damage.

Seven weeks after planting the leaves, differences among them included the following: leaves enlarged significantly in 96 seedlings and became greatly enlarged in 35; leaves became yellow in 10 cases, pale green in 33, and dark green in 162. Swelling of the petiole varied from none to an increase in diameter of about tenfold. Sixty-two seedlings had very enlarged petioles. Production of storage roots varied from 0 to 15 per leaf. Most of the storage roots were long and narrow, but some were thickened up to 1 cm. Only 24 of the seedlings regenerated sprouts.

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Changes in Phosphoenolpyruvate Carboxylase and Ribulose-1,5-bisphosphate Carboxylase in *Solanum tuberosum* L. as Affected by Root Zone Applications of CO_2[1]

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Additional index words. phosphoenolpyruvate carboxylase, ribulose-1,-5-bisphosphate carboxylase, CO_2 enrichment.

Abstract. **Changes in phosphoenolypruvate carboxylase (PEP-Case) and ribulose-1,5-bisphosphate carboxylase (RUBP-Case) were evaluated in potato plants following CO_2 enrichment of the root zone. There was a significant increase in PEP-Case activity in the roots of CO_2-treated plants between 3 and 12 days following CO_2 enrichment. Both PEP-Case and RUBP-Case in the leaves showed no significant change following CO_2 enrichment of the root zone. Of 4 additional species tested, potato roots had the greatest PEP-Case activity.**

Short-term CO_2 enrichment of the root zone of potato plants enhances overall dry matter content (1) with the beneficial effect persisting for weeks after treatment. This agrees with other observations of increased dry matter content following CO_2 enrichment of the root zone (11, 12, 13). $^{14}CO_2$ applied to the root zones of potato plants is fixed into malate (3), presumably via phosphoenolpyruvate carboxylase (10). In addition, $^{14}CO_2$ applied to the roots can be translocated to the top of the plant (3) where it could be utilized by ribulose-1,5-bisphosphate carboxylase to suppress photorespiration. Potato plants are able to incorporate CO_2 by phosphoenolpyruvate carboxylase (PEP-Case) for entry into the tricarboxylic acid cycle or for amino acid synthesis (6). Fixation of CO_2 by plant roots has been reported in barley (14, 15, 16), onions, and soybean roots (8). Recently, it has been shown that soybean roots and nodules possess an active system for fixing CO_2 (7). It has been shown by Triplett et al. (17) that malate originating in roots of wheat is transported in the xylem to the leaves.

The objective of this study was to determine if root zone applications of CO_2 altered the activities of PEP-Case and RUBP-Case in potato plants. In addition, 4 other species were surveyed to compare enzyme activities with potato plants.

Potato plants were grown as reported by Arteca et al. (1). Peas (*Pisum sativum* L.), beans (*Phaseolus vulgaris* L.), oats (*Avena sativa* L.), and corn (*Zea mays* L.) were grown in vermiculite and watered weekly with full-strength Hoagland solution (9). All plants were 4 weeks old and were grown in a growth chamber at 16 hr light (21.1°C) and 8 hr dark (15.6°). For the time course experiment, 17-day-old potato plants were treated with 20.0% CO_2 (21.0% O_2, with the balance N_2) for 10 hr (2).

RUBP-Case and PEP-Case crude enzyme preparations were obtained by grinding leaf or root tissue in a chilled mortar and pestle. The grinding buffer consisted of 0.1 M K-phosphate (pH 7), 10 mM $MgCl_2$, 20 mM 2-mercaptoethanol, 0.2 mM EDTA, and 16 g/liter PVP (4). The homogenate was centrifuged at 10,000 *g* at 4°C for 20 min after which the supernatant was recovered for enzyme assays.

For the ribulose-1,5-bisphosphate carboxylase assay we followed procedures outlined by Chu and Bassham (5). The reaction mixture contained 25 mM Tricine (pH 8), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 2.5 mM RUBP, 0.05 mM NADPH, 1 mM FDP, 50 mM $NaH^{14}CO_3$, and 5 to 25 µl of the enzyme extract.

The phosphoenolpyruvate carboxylase activity was assayed according to the procedure outlined by Williams and Kennedy (18). The reaction mixture contained 25 mM Tricine (pH 8.3), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 15 mM PEP, 15 mM sodium L-glutamate, 1.25 µM $NaH^{14}CO_3$ (specific activity 0.1 mCi/mMole) and 5 to 25 µl of enzyme extract.

Of the 5 species surveyed, potato roots had 3–4 times more PEP-Case activity, while in the leaves there were no significant differences among the C_3 species. The C_4 plant, corn, had much greater activity in the leaves as expected (Table 1). In addition, potato plants had the highest PEP-Case ratio (root and leaf) of all plants. The activities of RUBP-Case were quite similar in the C_3 species, with the C_4 plant having the lowest activity (Table 1).

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Table 1. PEP-Case and RUBP-Case activity in plants. Values expressed as the mean of 3 replications (3 plants per replication) ± SE.

Plants	PEP Case (μmoles/g dry wt·hr)		RUBP-Case (μmoles/g dry wt·hr)		
	Roots[z]	Leaves[y]	Roots/Leaves	Leaves[y]	RUBP-PEP
Potato	168.1 ± 11.3	163.5 ± 32.0	1.03	1039.8 ± 160.0	6.36
Pea	38.7 ± 5.4	143.3 ± 10.0	0.27	1055.9 ± 74.3	7.37
Bean	56.9 ± 2.0	137.0 ± 14.7	0.41	916.6 ± 82.1	6.69
Oat	16.6 ± 3.3	142.3 ± 29.6	0.12	1585.4 ± 295.6	11.14
Corn	51.2 ± 5.4	776.2 ± 38.5	0.07	511.0 ± 98.4	0.66

[z]Protein levels (mg/g dry wt) ± SE in the roots of potato, pea, bean, oat, and corn are 71.1 ± 9.7, 35.8 ± 5.0, 30.8 ± 2.7, 8.8 ± 1.8, and 22.6 ± 6.1, respectively.
[y]Protein levels (mg/g dry wt ± SE in the leaves of potato, pea, bean, oat, and corn are 309.2 ± 44.8, 217.4 ± 27.4, 246.0 ± 49.7, 176.3 ± 32.9, and 166.8 ± 41.1, respectively.

With respect to the effect of CO_2 treatment on enzyme activity, 2 days following CO_2 enrichment of the root zone, there was no significant difference between treated and control roots. Four days after CO_2 treatment, there was about 80% increase in PEP-Case activity and from this time on there was a highly significant difference in PEP-Case activity between treated and control roots (Fig. 1). Activities of PEP-Case and RUBP-Case in leaves showed no significant difference between control and treated leaves, although there appeared to be a trend for higher activities of each in leaves of treated plants (Fig. 2 and 3).

Results indicate that potato plants have a greater PEP-Case activity than other C_3 species tested and that CO_2 enrichment of the root zones of potato plants causes a stimulation of PEP-Case activity in the roots. The greater PEP-Case activity in potato roots suggests there is sufficient enzyme activity to utilize at least part of the existing CO_2 in the soil and, when higher levels of CO_2 are provided, enzyme activities increase either through enzyme synthesis or activation.

Immediately after CO_2 enrichment of the root zone, most CO_2 translocated to the top of the plant is dissolved in the xylem sap (3), possibly because roots do not contain sufficient PEP-Case activity to utilize the levels of carbon provided. However, after CO_2 treatment, there is an increase in PEP-Case activity and it is possible that there is a greater amount of fixation in the roots. Therefore, it appears that the initial benefit of CO_2 enrichment of the root zone could result from translocation of CO_2 to leaves where it is utilized by RUBP-Case. After a longer period, it is possible that there is an increase in the amount of assimilated carbon transported from the root to the top of the plant. Due to the increased PEP-Case activity after CO_2 treatment (Fig. 1), the additive effect of both the CO_2 dissolved in xylem sap, as well as carbon assimilated by the roots, could in part be responsible for the increase in growth reported previously (1).

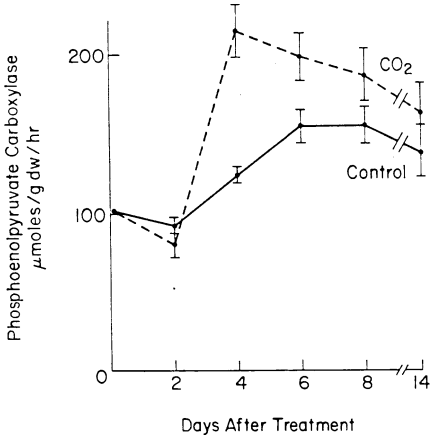

Fig. 1. Effects of CO_2 enrichment of the root zone (20.0% CO_2, 21.0% O_2 with the remainder N_2 for 10 hrs) on PEP-Case levels in the roots of potato plants over 14 days. Each point represents the mean of 3 replications (3 plants per replication) ± SE.

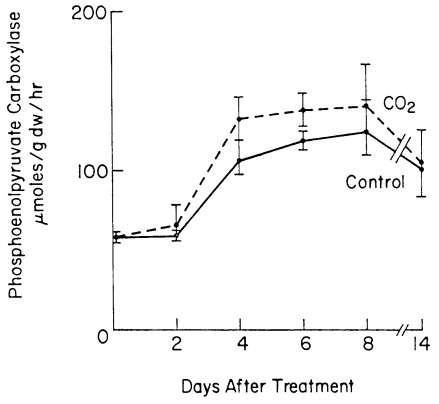

Fig. 2. Effects of CO_2 enrichment of the root zone (20.0% CO_2, 21.0% O_2 with the remainder N_2 for 10 hrs) on PEP-Case activity in the leaves of potato plants over a 14-day period. Each point represents the mean of 3 replications (3 plants per replication) ± SE.

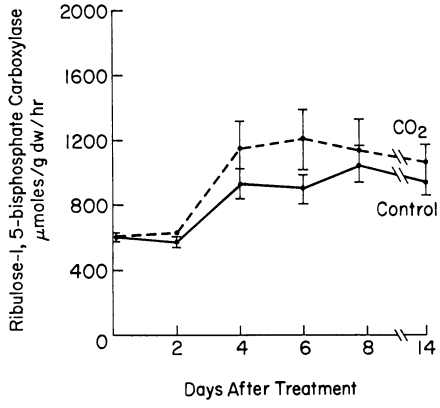

Fig. 3. Effects of CO_2 enrichment of the root zone (20.0% CO_2, 21.0% O_2 with the remainder N_2 for 10 hrs) on RUBP-Case levels in leaves of potato plants over a 14-day period. Each point represents the mean of 3 replications (3 plants per replication) ± SE.

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Pectic Enzymes in 'Long Keeper' Tomatoes[1]

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Additional index words. *Lycopersicon esculentum*, pectinesterase, polygalacturonase, fruit ripening

Abstract. **The changes in pectinesterase (PE) activity during maturation and ripening of 'Long Keeper' tomatoes (*Lycopersicon esculentum* Mill.) were similar to those of 'Marion' tomatoes. The pectinesterase activity was due to similar forms of this enzyme in both cultivars. In contrast, the polygalacturonase (PG) activity in ripe 'Long Keeper' tomatoes was much lower than that in ripe 'Marion' fruit. The activity in 'Long Keeper' fruit was due to 2 enzymes, one of which was similar to that found in 'Marion' fruit, but most of the activity appeared to be due to a new polygalacturonase. These differences may to a large extent account for the postharvest quality retention of the 'Long Keeper' cultivar.**

'Long Keeper' tomatoes harvested ripe can be stored successfully at cool conditions for 6 to 12 weeks (1). The superior keeping quality of this cultivar is reflected by the fact that it retains firmness better than most other cultivars. The loss of firmness in ripening tomatoes and other fruits is generally attributed to cell wall degradation by pectic enzymes (12). PG, in particular, appears to be closely associated with the process of fruit softening (5, 12). Wallner and Bloom (16) found that PG was the only enzyme in extracts of ripe tomatoes that solubilized isolated tomato cell walls. Because PG activity may be influenced by substrate esterification (7), cell wall degradation by PG may be controlled by PE, which hydrolyzes the methyl ester groups of pectin. Our study of 'Long Keeper' tomatoes was undertaken to determine whether their good retention of firmness is attributable to the concentrations and properties of PG and PE.

Seeds of 'Long Keeper' and 'Marion' tomatoes were obtained from W. Atlee Burpee Co., and George W. Park Seed Co., respectively. The plants were started in a greenhouse and then set out in field plots at Athens, Georgia. Fruit was harvested at various stages of maturity and ripeness and stored at -20°C.

The levels of PE and PG in 'Long Keeper' tomatoes were determined first. Fruit was separated according to 6 stages of maturity and ripeness (Table 1); 50 g of pericarp tissue from 6 tomatoes at each stage was homogenized with 50 ml of 2.0 M NaCl. The suspensions were adjusted to pH 6 with NaOH and stirred for 2 hr. They were then centrifuged at 9000 x g for 20 min and the sediments were discarded. Aliquots of the supernatants (5 ml) were dialyzed for 6 hr against 4 liters of 0.15 M NaCl and then for 12 hr against fresh 0.15 M NaCl to remove the soluble reducing sugars. All of the above steps were conducted at about 3°C.

PE was assayed by measuring the formation of free carboxyl groups by titration with base to maintain constant pH. The reaction mixture consisted of 10 ml of 1% pectin (pH 7), 40 ml of 0.125 M NaCl, and 0.5 ml of dialyzed tomato extract. The volume of 0.004 N NaOH required to maintain the pH at 7 was determined with an automatic titrator. A unit of PE activity is defined as that amount that catalyzed the release of 1 μmole of carboxyl groups in 10 min at 25°C.

PG was assayed by measuring the formation of reducing groups. The reaction mixture consisted of 0.5 ml of 1% polygalacturonic acid (PGA I) (pH 4.7), 0.2 ml of 1.0 M NaCl, and 0.5 ml of tomato extract. A blank for each sample was prepared by heating the extract 5 min in a boiling water bath before the addition of the substrate. The solutions were incubated at 37°C for 1 hr, and then 1 ml of each was analyzed for reducing groups by the arsenomolybdate method (8). A unit of PG activity is defined as that amount that catalyzed the release of 1 μmole of reducing groups in 1 hr. The substrates (pectic acid, PGA I, PGA II, and PGA III) for PG were prepared and purified as described earlier (9). The average degrees of polymerization for pectic acid, PGA I, PGA II, and PGA III were 201, 79, 20, and 13, respectively.

Table 1. Pectinesterase and Polygalacturonase Activities in 'Long Keeper' and 'Marion' Tomatoes at Various Stages of Maturation and Ripeness.

	PE (units/ml)		PG (units/ml)	
Stage	Long Keeper	Marion	Long Keeper	Marion
Green, 2–3 cm	58	50	0.03	0.04
Green, 4–5 cm	73	65	0.04	0.04
Green, >6 cm	137	153	0.07	0.05
Turning color	154	139	0.14	0.45
Medium ripe	130	137	0.61	8.7
Ripe	138	126	0.83	11.6

During maturation of 'Long Keeper' tomatoes PE activity increased and then peaked at the onset of color change (Table 1). The pattern of changes in PE for 'Marion' tomatoes was similar to that for 'Long Keeper' tomatoes, with comparable levels of activity in all 6 stages. As in other cultivars of tomatoes (5, 15), PG activity was negligible in developing fruit of 'Long Keeper' but increased concomitantly with ripening (Table 1). The activity increased much more markedly in ripening 'Marion' tomatoes. In ripe fruit, the PG activity was 14 times as high in 'Marion' as in 'Long Keeper' tomatoes.

Multiple forms of both PE and PG have been found in tomatoes (3, 10, 11). Therefore, we next wanted to determine the isoenzyme components in 'Long Keeper' fruit. Extracts for this phase of the study were prepared by homogenizing 500 g of ripe tomato tissue with 500 ml of 2.0 M NaCl. The homogenate was adjusted to pH 6, stirred for 2 hrs, and then centrifuged. The supernatant solution was made 80% saturated with respect to ammonium sulfate. The precipitated proteins were collected by centrifugation, dissolved in 25 ml of 0.15 M NaCl, and dialyzed against 0.15 M NaCl. The dialyzed extract was centrifuged, concentrated to 25 ml by ultrafiltration with an Amicon PM-10 membrane, and applied to a 5- x 70-cm column of

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