PN-ABH-102

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BEN-GURION UNIVERSITY
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Beer-Sheva, Israel

באר־שבע, ישראל

PN-ABH-102
ISN 69915

THE INSTITUTES FOR APPLIED RESEARCH
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COOPERATIVE ARID LANDS AGRICULTURAL
RESEARCH PROGRAM
UNITED STATES-EGYPT-ISRAEL
NEW CROPS FOR ARID LANDS:

GUAYULE - A POTENTIAL SOURCE OF NATURAL RUBBER

Eighth Annual Report
March 1989-February 1990

DAVID MILLS, ALIZA BENZIONI and MEIR FORTI

The Institute for Agriculture & Applied Biology

Supported by U.S. Agency for International Development
Bureau for the Near East
(File no. 8644221)

Beer-Sheva, Israel

March 1990

PREFACE

This report for 1989-90 is the last in the "Industrial Plant" section of the CALAR program. The emphasis during the eight years of activity in this section was on guyaule, a potential source of natural rubber. Here we report data collected during the last four years in the course of the irrigation trial, most of it not presented in the previous reports. The data pertain to the effect of water stress as well as season on the *in situ* accumulation of rubber, the fixation, translocation and incorporation of ^{14}C (supplied as $^{14}CO_2$), and finally the potential of branches to synthesize rubber (in terms of [^{14}C]acetate incorporation into rubber).

Acknowledgements

We would like to thank Ms. S. Avni for her excellent technical work, Mr. I. Knafo who carried out his M.Sc. thesis in the framework of this project, and finally Ms. A. Sen for styling this report.

EFFECT OF WATER STATUS AND SEASON ON THE PRODUCTION OF RESIN AND RUBBER IN GUAYULE

ABSTRACT

To establish whether water stress and cold temperatures stimulate rubber biosynthesis in guayule (*Parthenium argentatum*), a drought-resistant rubber-producing shrub, two-year-old plants grown under field conditions in the Negev desert of Israel were subjected to different irrigation regimes. Water status was assessed by measuring relative water content (RWC) and the temperature differential between air and leaf. Allocation of photosynthates to stems and leaves and incorporation into rubber and resin was studied by exposing undetached plant tips to $^{14}CO_2$ for a 1-h pulse followed by a 24-h chase. The stem rubber synthesis potential was determined by recording [^{14}C]acetate incorporation into rubber in detached branch slices. In addition, rubber and resin were extracted from tissues to determine their concentrations. *In situ* rubber concentrations were usually high at the end of winter and low in summer. The rubber concentration of the plants was somewhat higher in the low irrigation treatment than in the high. Both ^{14}C translocation and the rubber synthesis potential were maximum in the winter months. In summer the translocation of photosynthates into stems was inversely related to plant RWC, incorporation of ^{14}C into rubber and the potential of stems to synthesis rubber being high in water stressed plants and low in well irrigated plants. Resin synthesis was found to be equally unaffected by water stress and by season. Though the rubber synthesis potential is much increased by water stress, actual rubber accumulation is limited by translocation and overall reduction in biomass yield.

Key words - Biomass production, ^{14}C incorporation, ^{14}C translocation, [^{14}C]acetate incorporation, irrigation, *Parthenium argentatum*, relative water content, resin, rubber, season.

INTRODUCTION

Rubber accumulation in the branches and roots of guayule, a perennial desert shrub, is the end result of many processes. Chief among these are branch and root biomass production, synthesis and translocation of biochemical intermediates for polyisoprene synthesis, and induction and activation of enzymes like rubber transferase that enhance rubber synthesis. These processes are affected and partially regulated by several, occasionally conflicting environmental conditions. There is good evidence that low temperatures trigger the induction or activation of rubber synthesizing enzymes, particularly rubber transferase (Backhaus and Bess 1986, Gilliland et al. 1985, Goss et al. 1984). In addition, it has been established that water stress increases rubber concentration (Benedict et al. 1947, Hammond and Polhamus 1965). On the other hand, both the rate of root and branch biomass production and the rubber yield are directly correlated with the rate of vegetative growth and are maximal under good water status and warm temperatures (Benzioni et al. 1989, Bucks et al. 1984, 1985, Ehler and Nakayama 1984).

Very little is known about the nature of the intermediates involved in rubber synthesis, where they accumulate, and the factors regulating their translocation to the rubber synthesizing parenchymal cells. Some work has been done on the role of photorespiration in supplying intermediates for rubber synthesis using detached young branches (Reddy et al. 1986, 1987). It was found that a significant proportion of ^{14}C (about 17%) was fixed in serine, glycine and glycolate during a three minute $^{14}CO_2$ pulse. Inhibition of the photorespiratory CO_2 flow inhibited rubber synthesis, indicating a possible role of photorespiration intermediates in rubber synthesis. However, no data are available on the effects of environmental conditions on translocation of photosynthates to different sinks and products. In the present study the effects of water stress and season on some aspects of photosynthate partitioning into rubber producing sinks and notably into the rubber fraction are investigated.

MATERIALS AND METHODS

Field procedures

The experiment was conducted on two- to three-and-a-half year old plants of USDA line 11604 growing in Omer, near Beer-Sheva, Israel. The water doses applied were 250, 450, 650 and 1100 mm per annum (dry, medium, wet and very wet, respectively). Irrigations were scheduled every third or fourth week (every ten days for the very wet treatment) from April to October so that all but the very wet treatment subjected the plants to regularly recurring water stress. Plants were irrigated using one drip line per row, one dripper supplying 2 liters per hour per 50 cm. Other field procedures as well as determination of RWC and temperature difference between canopy and air are described in Mills et al. (1988) and Benzioni et al. (1989).

Photosynthetic labeling partitioning

Several plants were selected at random in each of three irrigation treatments (dry, medium and wet) and $^{14}CO_2$ of 0.185 KBq ($NaH^{14}CO_3$ 2.15 KBq μmol^{-1} Amersham) was supplied to one branch per intact plant. Each intact branch tip (4-5 cm long with at least one fully developed leaf) was enclosed in a transparent cell and exposed to $^{14}CO_2$ generated by injecting a few drops of concentrated sulfuric acid. After exposure to $^{14}CO_2$ for one hour (usually at 7 AM) the branch tip was further chased for 24 hours. A section of branch about 25 cm in length (including the tip portion exposed to $^{14}CO_2$ and three side branches) was harvested and divided into pulsed leaf (1), pulsed stem (2), main stem (9 & 10), side branches (4,6 & 8), and leaves (3,5 & 7) (Fig. 1). The different organs were dried at 70°C for 48 h, weighed, frozen with liquid nitrogen, and ground to powder in a mortar. Individual samples of 10 mg were dissolved in a scintillation vial in: 0.15 ml water, 0.30 ml of a tissue solubilizer (Soluene-350, Pacard), and 0.70 ml toluene. The vials were incubated at 55°C overnight, then discolored by adding 0.1 ml isopropanol and 0.1 ml H_2O_2. Thirty minutes later scintillation fluid (toluene/triton) and 0.1 ml glacial acetic acid were added in order to decrease chemoluminescence and radioactivity was determined in a liquid scintillation counter (Beckman LS 1701). Samples of the main stem portion weighing 200 mg each were extracted twice with acetone and twice with cyclohexane by a procedure described elsewhere (Benzioni et al. 1989) and the radioactivity in these fractions was determined.

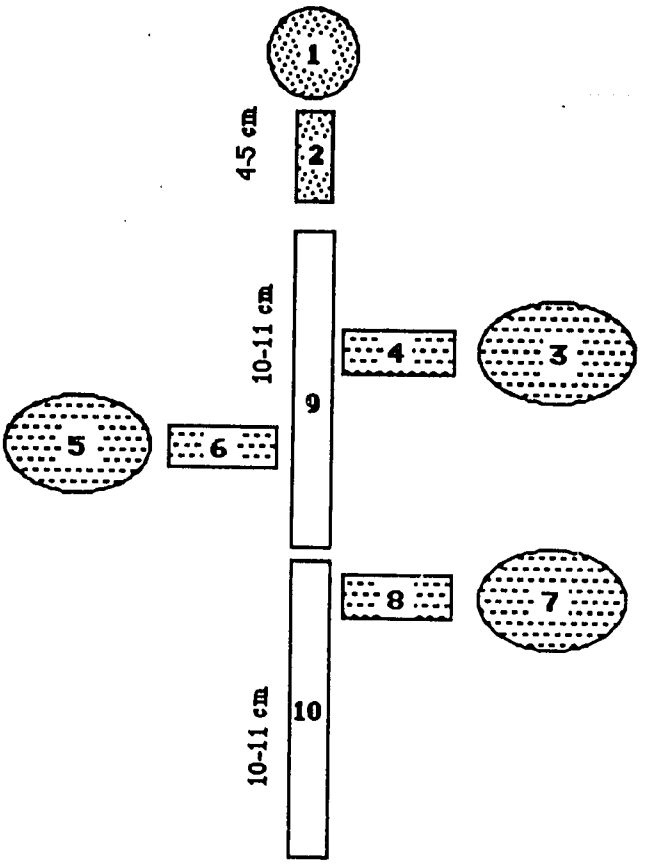

Fig. 1. Diagram representing a branch used in the $^{14}CO_2$ translocation study. The tip, consisting of leaves (1) and stem (2), was exposed to the pulse. The upper (9) and lower segments (10) of the adjacent main stem, the three side branches (4,6,8), and the leaves (3,5,7) were analyzed separately.

[^{14}C]acetate incorporation

The procedure for [^{14}C]acetate incorporation was adapted from Marrea et al. (1985). Two branches were harvested from each of five plants per treatment, the same plants being used throughout the experiment (July 1987-March 1989). Leaves were removed from the branch tip over a length of 5 cm and the branches washed in 1% hypochlorite for 15 minutes, rinsed in distilled water, and cut into thin (0.5-1.0 mm) slices. The slices were placed in a phosphate buffer solution (0.1 M, pH 6.5) and mixed. Three 0.5 g fresh weight samples were transferred into 4 ml of incubation

medium containing: 0.1 M phosphate buffer pH 6.5, 0.4 mM acetate, 0.92 Kbq 2-[^{14}C]acetate, 0.4 mg penicillin, and 0.4 mg ampicillin. The hypochlorite and antibiotic treatments were applied to prevent bacteria from developing in the medium and consuming part of the labeled acetate. Incubation took place on a shaking bath at 26°C under light (50 $\mu Em^{-2}s^{-1}$) in stoppered 25 ml erlenmeyer flasks. Two ml of hyamine was placed in a central well (Knots) in each erlenmeyer to trap $^{14}CO_2$ evolved during incubation. An incubation time of 3 h was found to assure a high enough uptake of [^{14}C]acetate for a fall of no more than 10% in the acetate concentration of the medium. Radioactivity in the medium before and after incubation and radioactivity in the hyamine fraction were determined in order to enable calculation of the recovery, which was between 90 and 95%. The reaction was stopped by decanting the incubation medium and washing the tissue with cold buffer and then twice with boiling ethanol, the resulting fraction being set aside for further analysis. The tissue was then homogenized in hot ethanol with an ultratorax homogenizer (Polytron model FT 10/35), centrifuged and extracted again in ethanol. All ethanol fractions were combined, their volume measured and the combined fractions sampled for determination of radioactivity. The tissue was further extracted twice in acetone and twice in cyclohexane as above (Benzioni et al. 1989). The combined acetone and cyclohexane fractions were placed in a scintillation vial and dried on warm sand in a hood, and subsequently scintillation solution was added and the radioactivity recorded. The remaining pellet was dried and weighed, after which samples of 20 mg were dissolved in a scintillation vial in soluene/toluene as described above for the dried powder. A sample of the sliced branch material was dried at 70°C for 48 h for determination of percentage dry weight. Total radioactivity incorporated into the tissue and the percentage in each fraction were calculated.

RESULTS AND DISCUSSION

Rubber, resin and biomass production

In a previous report (Mills et al. 1988) we described the effect of water status on rubber and biomass production in two-year-old guayule plants. Data collected since then and covering a period of four years are presented here. The changes in leaf RWC and canopy-air temperature difference recorded in the course of an irrigation cycle are shown in Fig. 2. The two parameters studied, both of which reflect the water status of the plant, were found to be in good correlation with the available soil moisture (Mills et al. 1988). While plants in the dry treatment exhibited signs of increasing water stress ten days after irrigation, plants in the wet treatment did so six days later (Fig. 2). In the medium water treatment water stress developed 10-13 days after irrigation (Mills et al. 1988). Thus stress was at once more severe and more prolonged in the dry than in the medium and high treatments.

Water stress affected both biomass production and *in situ* rubber and resin accumulation. Increasing water quantities were generally associated with low rubber concentrations (Fig. 3) and high resin concentrations (Fig. 4). On the other hand, biomass production was directly correlated with the amount of water supplied (Fig. 5). Thus, although the rubber concentration was somewhat higher in the dry treatment, the overall rubber yield was lower (Fig. 6), since the associated reduction more than compensated for higher rubber concentration.

During the first two years rubber accumulation in branches was distinctly higher in winter than in summer, the season of vigorous growth. This phenomenon was not discernible in the third year. The reason for this is not clear. Since rubber formed in the plant is not metabolized, rubber concentration in the tissue reflects its production over the entire life of the plant, and rubber production in a relatively short period (days and weeks) is masked by the rubber accumulated previously. Therefore we studied $^{14}CO_2$ and [^{14}C]acetate incorporation into rubber as a function of season and water status of the plants in order to assess short-term synthesis of rubber.

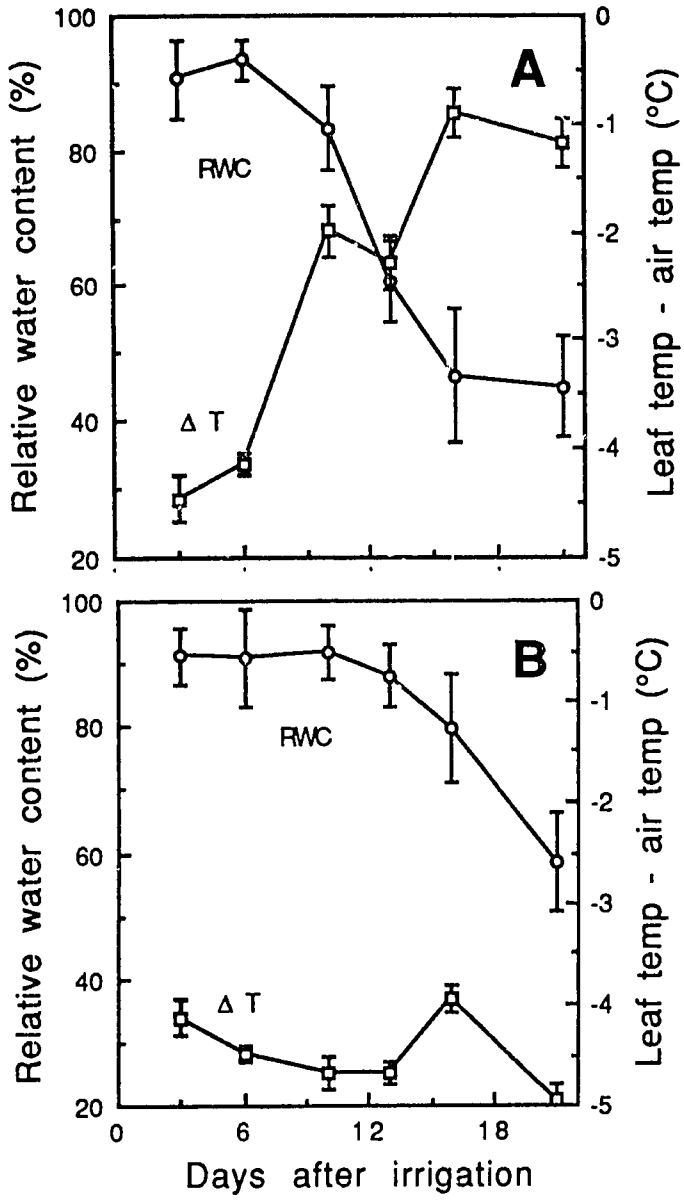

Fig 2. Relative water content (RWC) of leaves and temperature difference between plant canopy and air during an irrigation cycle in the dry (A) and the wet water treatment (B).

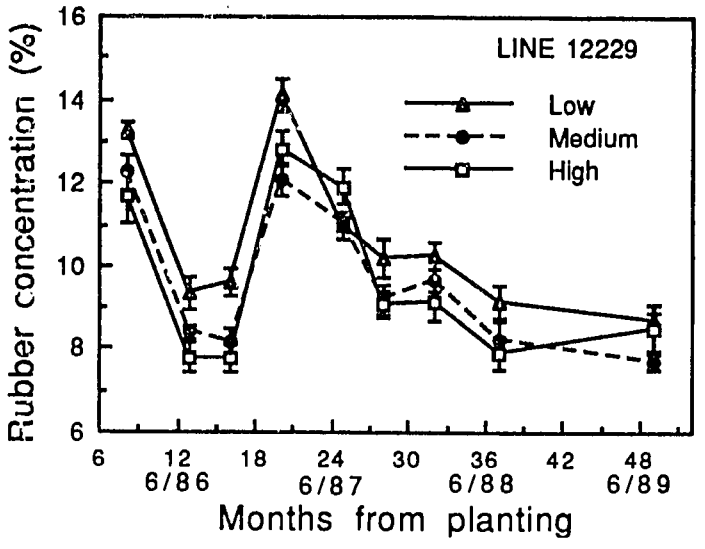

Fig. 3. Effect of irrigation on rubber concentration in guayule plants of line 12229.

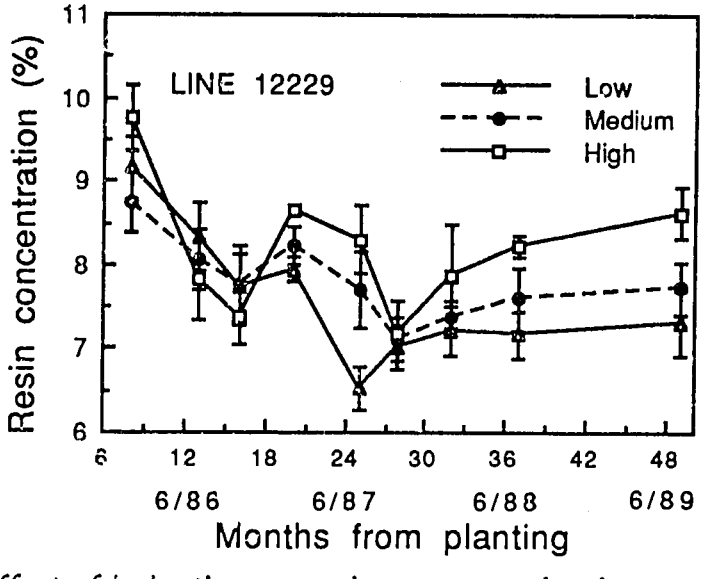

Fig. 4. Effect of irrigation on resin concentration in guayule plants of line 12229.

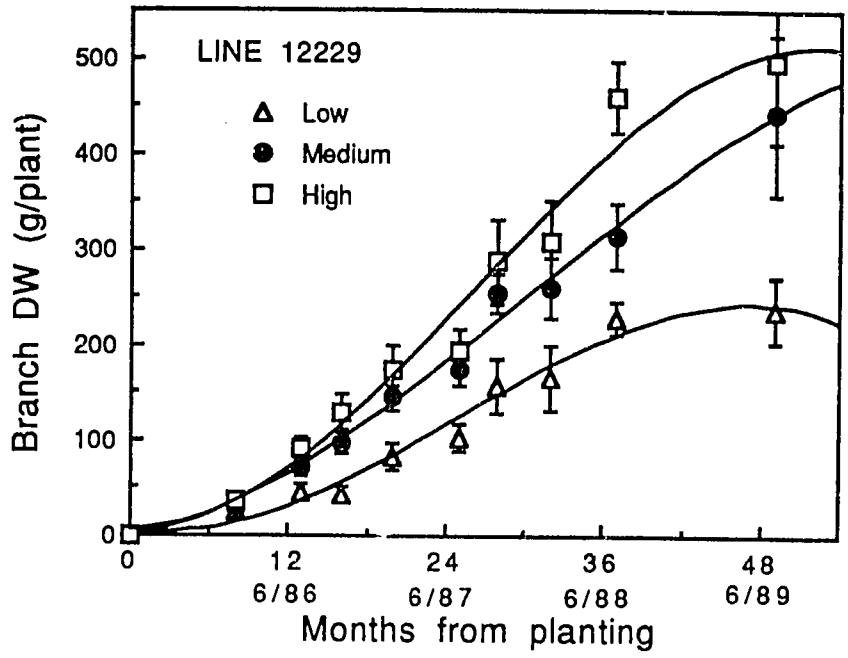

Fig. 5. Effect of irrigation on biomass production in guayule plants of line 12229.

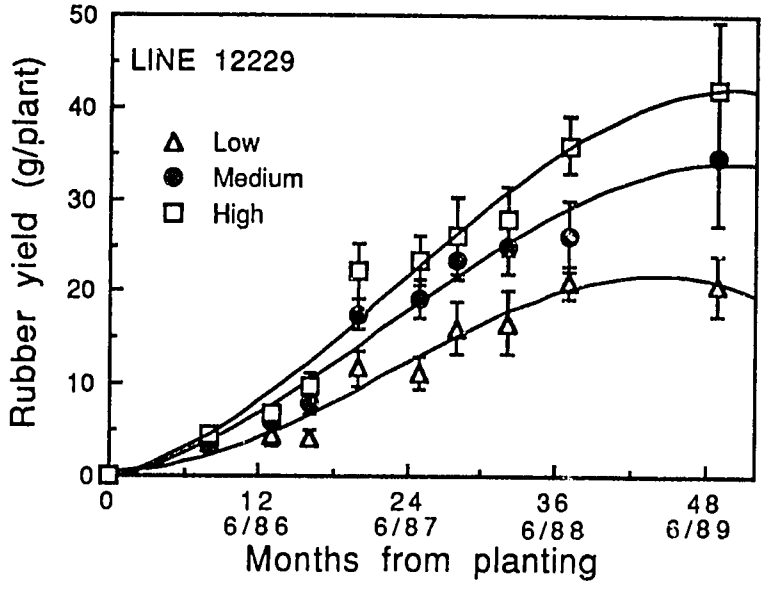

Fig. 6. Effect of irrigation on rubber yield in guayule plants of line 12229.

Photosynthetic labeling partitioning

Fixation of $^{14}CO_2$ in the stem tip (4-5 cm) and the translocation of ^{14}C to side branches and to the main stem was studied in intact guayule plants in the field under different water status and climatic conditions. Irrigation regime, season, and water availability had no perceptible effect on the amount of $^{14}CO_2$ fixed per mg dw of pulsed stem tip (Tab. 1). However, both season and water status affected the percentage of fixed ^{14}C exported from the pulsed tip during a 24-h chase; translocation was maximum in winter (about 28%), at which time it was similar for the two irrigation treatments, and distinctly smaller in summer. During the irrigation cycle the percentage of ^{14}C translocated from the fixing branch tip dropped from 14-18% three days after irrigation to 2-3% seventeen days later, the decrease being particularly sharp in the dry treatment (Tab. 1). The higher translocation in winter compared with summer could be attributed to reduced sink activities in the fixing branch associated with dormancy (Wardlow 1968), while the drop in translocation with increasing water stress may be a result of reduction of water influx into the phloem, which is thought to play a role in driving translocation (Brevedan and Hodges 1973, Plaut and Reinhold 1967).

Most of the exported ^{14}C stayed in the main stem, notably about 94% in winter and around 90% in plants just after irrigation in the summer. Later in the irrigation cycle the proportion retained in the main stem dropped to 30 and 70% in the dry and wet treatments respectively. The same pattern is reflected in the disintegration per minute (DPM) per unit weight, which dropped from 207 and 358 three days after irrigation to 31 and 162 seventeen days later for the dry and wet treatments, respectively. In general leaves and side branches imported only a small fraction of the total fixed ^{14}C. The amount of fixed ^{14}C imported did not decrease with depletion of the soil water, resulting in a relative increase in the percentage translocated from the fixing tip during each irrigation cycle. There was no correlation between RWC and ^{14}C translocation into leaves and side branches, but falling RWC was accompanied by a sharp decline in ^{14}C translocation into the main stem (Fig. 7).

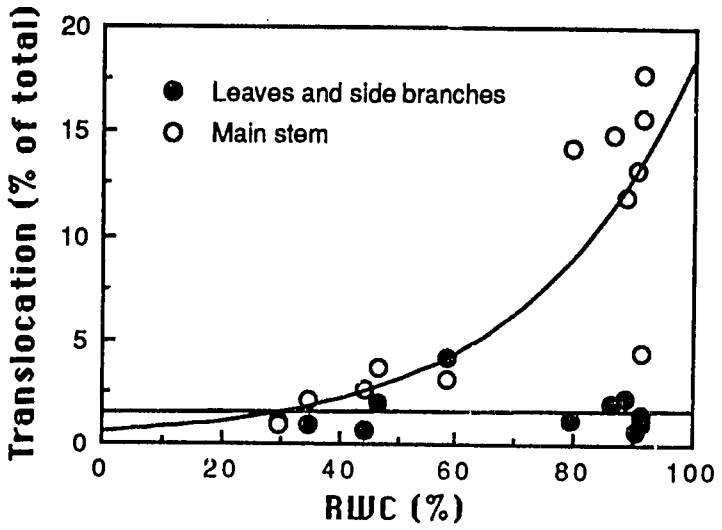

Fig. 7. Percent ^{14}C translocated out of the total $^{14}CO_2$ fixed after a one hour pulse followed by a 24-h chase as a function of leaf RWC. Numbers represent means for 3 to 4 plants for each of three irrigation treatments during two irrigation cycles in July and August 1987. The main stem comprises fractions 9 &10, while leaves and side branches comprise fractions 3 to 8 (see Fig. 1).

While this effect would appear to be inconsistent with the assumption that water stress enhances rubber synthesis and accumulation in the main stem, it is possible that a larger fraction of the translocated photosynthate is allocated to rubber under stress conditions. In order to test this possibility the partitioning of translocated ^{14}C into rubber and resin fractions was studied (Fig. 8). Significant (P=0.001) differences were found between the treatments, with higher amounts of ^{14}C going into rubber and resin in the dry treatment. While the drop in RWC during the irrigation cycle seems to have increased the amount of ^{14}C allocated to rubber, this effect is not statistically significant (Fig. 8).

[^{14}C]acetate incorporation

The effect of season and water status on the activity of the rubber synthesizing enzymes was studied by a nondestructive radiochemical method using detached branch slices. This method determines the rubber producing potential of the plants in accordance with Macrae et al. (1985) in terms of the ability of the plant material to incorporate excess labeled precursor into rubber under specified conditions. During

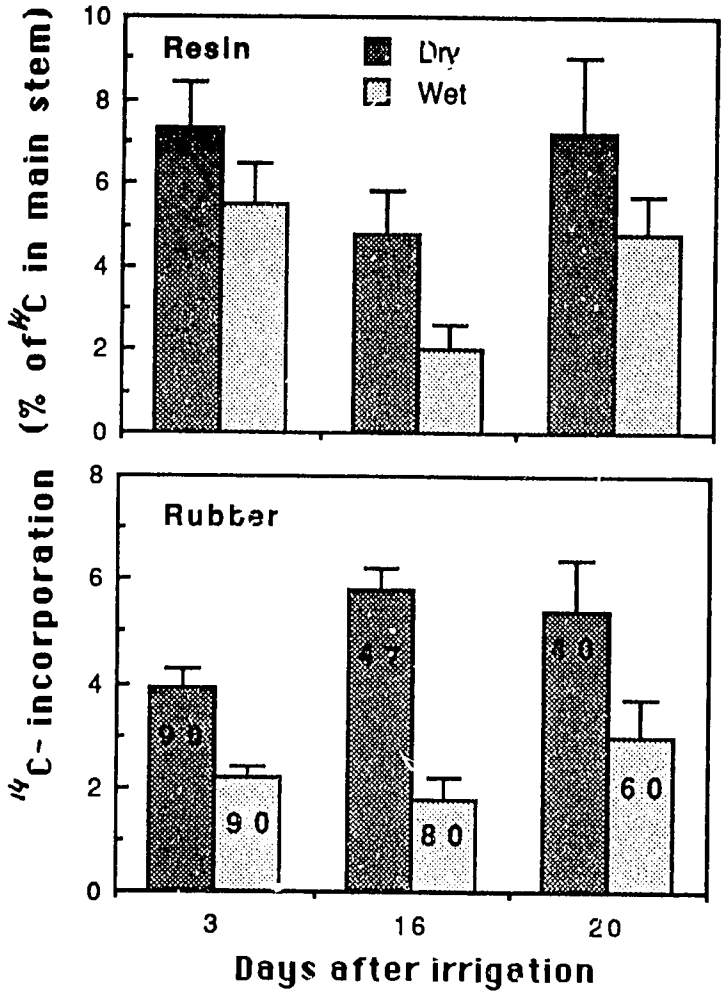

Fig. 8. Partitioning of photosynthetic ^{14}C between the resin (acetone) and rubber (cyclohexane) fractions in branches. The numbers on the bars represent the % RWC of leaves at the time of the experiment (July - August 1987). Values are means ± SE from 7 plants (3rd day after irrigation) and from 3 and 4 plants (16th and 20th day after irrigation).

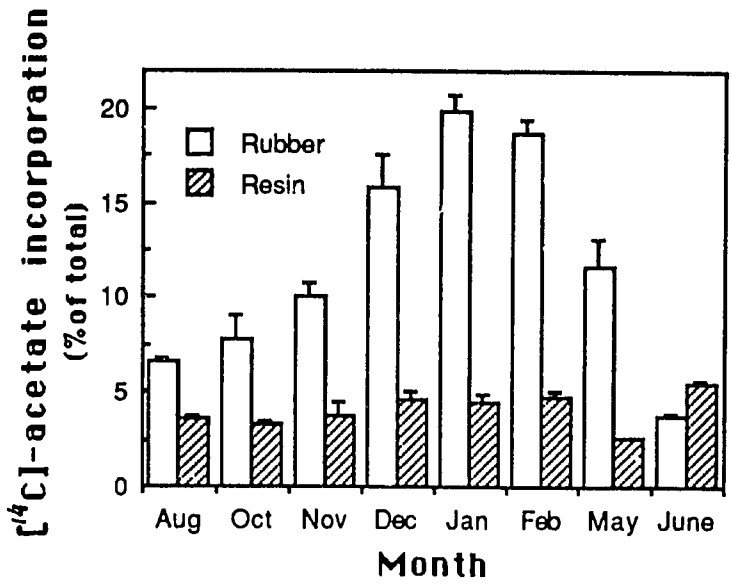

Fig. 9. Seasonal variation in the incorporation of [^{14}C]acetate into the resin and rubber fractions in plants of the wet treatment. Values are means for 3 to 6 replications, tissue being taken from 2 branches of each of 5 plants. The experiment was conducted over a period of one and a half years from October 1987 to May 1989. RWC of the plants was 85 to 95%.

the year [^{14}C]acetate incorporation into rubber increased from about 4% (in plants shortly after irrigation) in summer to 20 and 26% in winter (Fig 9 and 10). No such rise was found to characterize acetate incorporation into resin (Fig. 9).

The irrigation treatments did not differ in their effect on [^{14}C]acetate incorporation into rubber during most of the winter, but plants from the very wet and wet treatments incorporated higher amounts of [^{14}C]acetate into rubber than plants from the dry treatment in some months (Fig. 10). The data recorded for acetate incorporation during the summer irrigation cycles indicate that water status is a decisive factor in the potential of the plants to synthesis rubber (Fig. 11). The drop in RWC in summer from around 90 to 60% was accompanied by an increase in [^{14}C]acetate partitioned into rubber from about 5 to 20% and more (Fig. 12). Therefore a clear effect of season and water status on the potential of branches to incorporate ^{14}C from [^{14}C]acetate into rubber can be demonstrated.

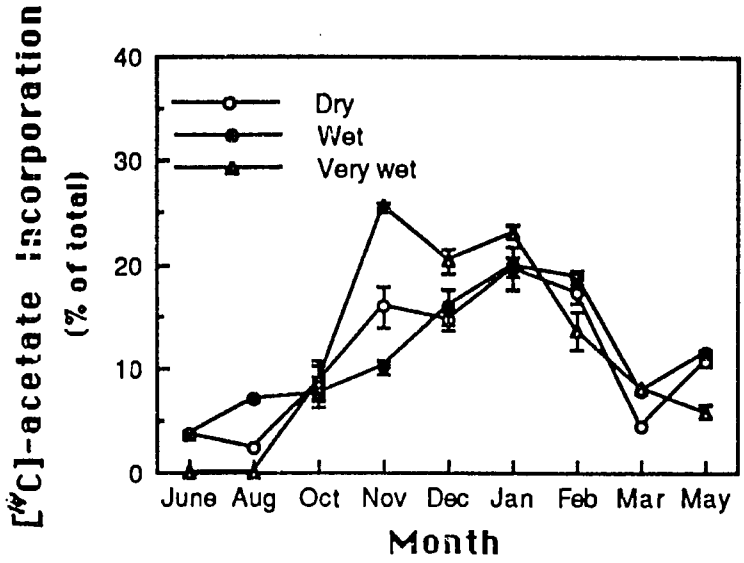

Fig. 10. Effect of irrigation regimes on the incorporation of [^{14}C]acetate into the resin and rubber fractions. Discs were pooled from 10 branches sampled from 5 plants per treatment. Values are means ± SE over 3 replications per treatment.

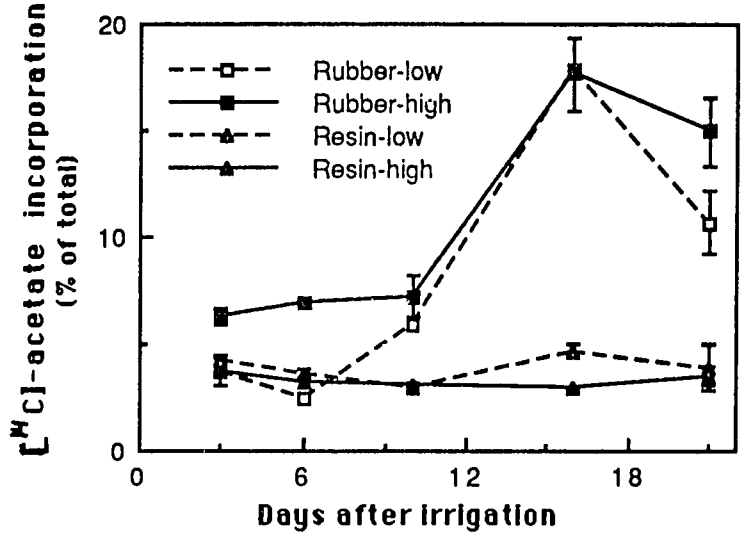

Fig. 11. Effect of time elapsed after irrigation on the incorporation of [^{14}C]acetate into rubber and resin for the dry and wet irrigation treatments (July 1987). Discs were pooled from 10 branches sampled from five plants per treatment. Values are means ± SE over 3 replications per treatment.

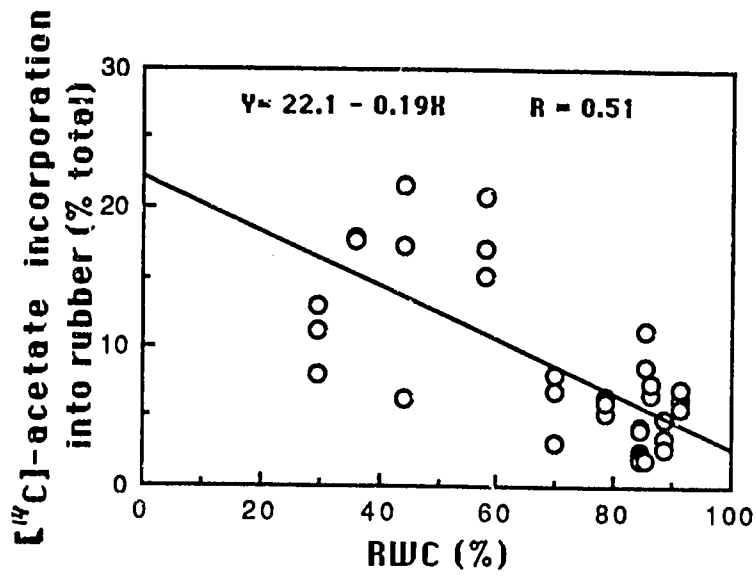

Fig. 12. Relationship between RWC in leaves and the incorporation of [^{14}C]acetate into rubber in detached branches. Values are means of 3 replications per treatment, each replication consisting of 10 branches sampled from 5 plants per treatment. Plants from the dry, wet and very wet treatment were used.

Thus rubber synthesis potential is not correlated with actual rubber synthesis in the plant, which is determined by many additional factors, including the nature of substrates in the translocate, the amounts of substrate available, competition with other sinks, and the different rates of synthesis of tissues capable of accumulating rubber. Furthermore from the results presented here and in the literature it is clear that water stress affects all the above mentioned factors, often in conflicting ways. While water stress inhibits growth and production of new stem tissue (Benzioni et al. 1989, Buck et al. 1984, 1985, Fangmeier et al. 1985) and the translocation of photosynthates to the stems (Tab. 1, Fig. 7), it enhances the amount of translocates incorporated into rubber (Fig. 3, 6 & 7). Moreover the severity and duration of stress play a role. To sum up, the overall effect of water stress on rubber accumulation is very hard to predict, and before any recommendations can be drawn up regarding stress regimes aimed at optimizing production, extensive further study will have to be carried out.

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