

Seasonal variations of leaf water potential and growth in fertigated carob-trees (*Ceratonia siliqua* L.)

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Abstract

Variations of predawn and midday leaf water potential and relative growth rates were studied in mature carob trees (*Ceratonia siliqua* L. cv “Mulata”) submitted to a fertigation experiment. Three levels of irrigation were tested: 0%, 50% and 100%, based on daily standard evaporation values. For each irrigation level two nitrogen amounts were applied - 21 and 63 kg N ha⁻¹ year⁻¹ as ammonium nitrate. The experiment was run between July 91 and August 1993. Measurements of leaf water potential and absolute branch length increments were made at monthly intervals, during the entire experimental period or during seasonal growth, respectively. Leaf water potential was related to soil volumetric water content, maximum and minimum air temperature and daily evaporation. Predawn leaf water potentials were always higher than -1.1 MPa. Midday leaf water potential values presented very large seasonal variations and very low values independent of treatments. The low leaf water potentials observed for the fertigated trees during summer, suggest that this parameter may be related not only to the evaporative demand but also to growth investment. The amount of fertigation was positively correlated with vegetative growth increment and fruit production. Practical implications for irrigation schedules of leaf water potential patterns together with drought adaptation mechanisms of carob tree are discussed.

Introduction

Carob tree (*Ceratonia siliqua* L.) is known to possess several types of drought resistance mechanisms (Lo Gullo and Salleo, 1988; Nunes et al., 1989, 1992; Salleo and Lo Gullo, 1989). Like in other Mediterranean species, with long-lived leaves (Diamantoglou and Mitrakos, 1981) and an extended flowering period (Martins-Loução and Brito de Carvalho, 1991) mechanisms have evolved that optimize water, carbon and nitrogen use for reproduction. It is also a high value cash crop and a valuable resource for reforestation to prevent erosion processes in marginal lands. However, some agronomical practices need to be improved to guarantee optimization as well as reliable carob productivity and maintain the economical viability of this species.

Information relating yield and rainfall is scarce. Marti and Caravaca (1990) presented some references which indicate that a rainfall range of 400–500 mm per year is needed to obtain a good fruit production.

An important attempt for introducing irrigation practices in carob plantations in marginal lands was made in the Negev Desert (Israel) by Herwitz et al. (1988). They showed that the use of runoff water on a micro-catchment system could sustain carob-trees having leaf surface areas over 13 m² and Merwin (1980) reported the use of runoff water and drip-irrigation of carob-trees in Mexico.

Since Correia and Martins-Loução (1990) showed the importance of drip-irrigation during plantation of young carob in Portugal, in 1991 a study was initiated concerning the possibilities of improving growth and yield in existing mature orchards through a fertigation system. It is, thus, necessary to obtain data

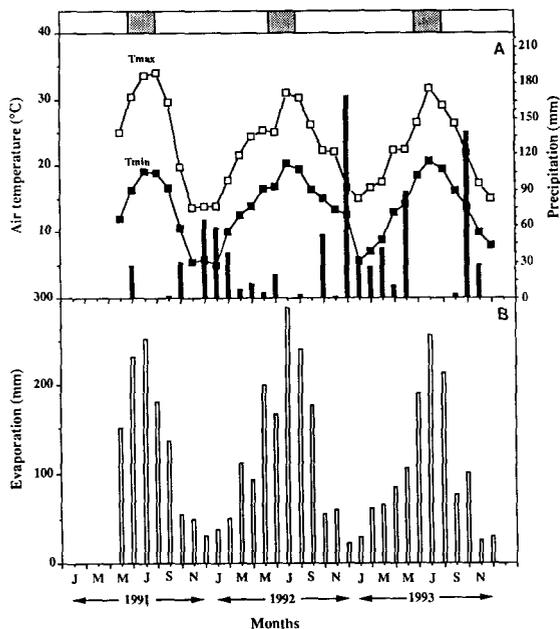


Fig. 1. Seasonal variation of atmospheric conditions at the experimental field. Fertigation periods are indicated by the shade bars in the upper part of the graph. A. Minimum and maximum air temperature and precipitation. B. Air evaporation.

concerning the relationship between carob production and tree water status in order to optimize the establishment of irrigation schedules. Several relationships have been found between atmospheric parameters and plant water status (Nel and Berliner, 1990; Peñuelas et al., 1992) mainly to optimize irrigation regimes and prevent water stress. Leaf water potential is one of the most widely studied plant parameters concerning those relationships.

In this work we investigate if leaf water potential is a good parameter for the diagnosis of the factors affecting soil-plant-atmosphere continuum in trees submitted to different fertigation regimes. The relationship between nitrogen gain and water status was also studied in order to verify if there were any variations in tree response due to nitrogen effects on the balance between carbon gain and water loss.

Materials and methods

Experimental site and soil parameters

The orchard used for the experiment presented 20–30 years old mature trees (cv. “Mulata”) with 2–3 m height and 14.9 ± 0.4 cm trunk diameter, established

in a 12×12 m spacing (69 trees ha^{-1}). It is located in the southern region of Portugal ($37^\circ 13' \text{ N}$; $7^\circ 28' \text{ W}$). The soil is a sandy loam, with a large amount of gravel throughout the profile, with low levels of organic matter (1.5%), C/N (7–11) and poor in N (0.15%). Annual rainfall, temperature and evaporation of the site are shown in Figure 1, showing characteristics of a typical Mediterranean climate (Mitrakas, 1981).

The field trial consisted of three irrigation (I) levels (0%, 50% and 100%) based on water loss by evaporation (E_{pan} measured with a class A pan; Doorenbos, 1976) according to the formula $I = E_{\text{pan}} \times \text{CF}$. The conversion factor, CF, (8.4 m^2) was calculated as the average of the projected canopy area of all the trees in the experiment. In that formula the cultural coefficient (K_c) was not used, since no K_c is known for carob. Two N-fertilizer levels were used: 21 kg ha^{-1} and 63 kg ha^{-1} . The N concentration was 0.3 kg tree^{-1} and 0.9 kg tree^{-1} , corresponding to 1.5 kg tree^{-1} and 4.5 kg tree^{-1} of total fertilizer, respectively. The fertilizer contained 15% calcium and 20.5% nitrogen, with equal parts of nitrate and ammonium. The fertilizer was applied once per year and spread all over the area under the canopy. The water was applied daily, during the morning through the dry season, between June and August of 1991, 1992 and 1993. The irrigation consisted of a micro-sprinkler system, one per tree and closed to the tree trunk, delivering 40 L h^{-1} , with a range of 360° . Border effects and runoff water are not expected due to the wide tree spacing and a small hill slope ($<10\%$). As the experimental trees are relatively small, considering their age, we also do not expect water uptake from adjacent irrigated trees.

A total of six treatments were tested. Each treatment consisted of 3 replicates of 4 trees in a total of 12 trees per treatment distributed in randomized design: 1.5/0, 1.5/50, 1.5/100, 4.5/0, 4.5/50 and 4.5/100 (N/water). On the 0% water level the fertilizer was applied two months before the other treatments in order to ensure dilution through the last rainfall. For the other water levels the fertilizer was applied at the beginning of the irrigation period (June).

Before irrigation three soil samples per treatment were taken with an auger under tree canopy at a distance of 60–80 cm from the trunk and always in north direction. The samples were taken at 30–40 cm depth, because “in situ” observations, in a well irrigated tree, indicated the presence of active roots at this depth. All the samples were taken during the early hours of the morning. They were weighed and dried in an oven for 48 h at 105°C for gravimetric water content.

Soil volumetric water content (VWC), in g cm^{-3} , was calculated using the gravimetric method, through the formula:

$$\text{VWC} = \left[\left(\frac{W_{\text{ms}} - W_{\text{ds}}}{W_{\text{ds}}} \right) \right] \times \text{bd}$$

where W_{ms} , W_{ds} and bd , are the weight of moist and dry soil and the bulk density, respectively. Bulk density used in the formula (1.37 g cm^{-3}) was obtained by taking an undisturbed block of soil with a metal cylinder.

Leaf water potential - LWP

Predawn and midday LWP were determined in the field with a Scholander-type pressure chamber in mature leaves taken from the south side of the canopy. In each treatment, the leaves used to measure LWP were taken off from 3 different trees. Three leaves per treatment were used for the measurements, which were done monthly during the irrigation season. Some measurements were also done during winter. Between dates the same trees were used. A wet filter paper was placed inside the chamber in order to minimize leaf water evaporation.

Meteorological data

Maximum and minimum air temperatures, relative air humidity and precipitation were recorded daily using a thermo-hygrograph and a raingauge placed within the orchard. Mean month temperature and evaporation were used in the regressions with LWP.

Branch length increment

At the beginning of the experiment (May 1991) 9 trees per treatment, homogeneous both in size and shape, were selected, and 8 branches on each tree were marked on the external side of the canopy. The terminal 100 cm of each branch was labelled and the subsequent shoot length increment of those branches were followed monthly all over the years. During winter time no growth was registered.

The absolute growth length increment was determined in order to evaluate the effects of fertigation treatments. Therefore the values shown were only reported to the growing season.

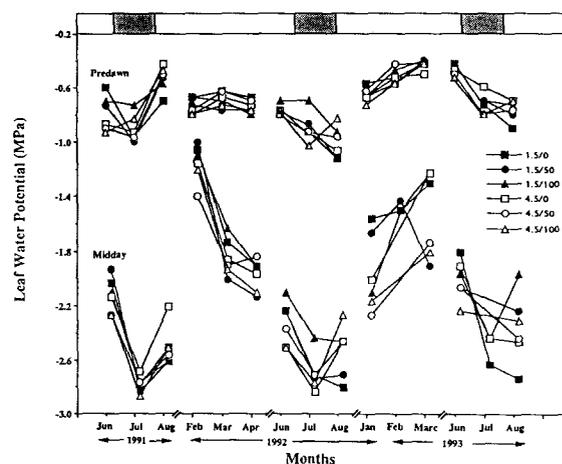


Fig. 2. Seasonal variation of predawn and midday leaf water potential for all treatments. The symbols referred as 1.5/0 – 4.5/100 means N/water application. Fertigation periods are indicated by the shade bars in the upper part of the graph. Each point is an average of 3 measurements per treatment.

Fruit production

Fruit was harvested in September of each year from 91 to 93 and expressed as kg per hectare.

Statistical analysis

For each measurement, differences among treatments were compared by analysis of variance, using F-test and Duncan's multiple range test (DMRT). "Water", "Nitrogen" and "Water \times Nitrogen" interactions were tested by the same procedure. Relations between predawn and midday leaf water potential and climatic variables, such as air temperature, evaporation and VWC were examined through linear regressions. All the data analysis were made with a computer program Statgraphics version 5.0 STSC.

Results

Meteorological data at the experimental station are shown in Figure 1. Figure 2 shows predawn and midday leaf water potential (LWP) between June 91 and August 93.

In spite of the small seasonal variation between and throughout years for the predawn leaf water potential values, significant differences during irrigation seasons were observed. The highest values were obtained for the well-irrigated treatments, in July 91: -0.73 MPa

Table 1. Significance levels of differences among treatments for predawn and midday LWP obtained from multifactorial ANOVA table using F-test. These data are related to Figure 2, however only the irrigation season was considered for each year. Significant at 95% (*) and 99% (**). Non-significant (ns)

Factors	Jun 91	Jul 91	Jun 92	Jul 92	Au 92	Jun 93	Jul 93	Au 93
LWP (Predawn)								
Water (W)	ns	*	ns	ns	*	ns	ns	ns
N	ns	ns	ns	**	ns	ns	ns	ns
N × W	ns	ns	ns	*	ns	ns	ns	ns
LWP (midday)								
Water (W)	ns	ns	*	ns	**	*	ns	**
N	ns	ns	ns	ns	ns	ns	ns	ns
N × W	ns	ns	ns	ns	ns	ns	ns	ns

and -0.83 MPa for 1.5/100 and 4.5/100, respectively (Fig. 2). No nitrogen (N) nor N × water interactions effects were registered. Only in July and August 1992 significant differences were observed between treatments, the water effect being more marked in August than in July (Table 1). During this month a positive interaction between nitrogen and water was registered as well as a greater nitrogen effect predominance. In 1993, predawn values obtained in summer were not statistically different, all the treatments showing slightly higher values than those of the previous year. Apparently, the same trend was obtained all over the year. This fact is probably associated with rainfall events between February 93 and the end of May (Fig. 1). No significant differences were observed in predawn LWP during winter and early spring, except on the first year when a N effect was detected (Fig. 2).

Midday LWP were low despite the irrigation levels (Fig. 2). Significant differences between treatments were not evident, although seasonal variations were observed. However statistical differences (Table 1) were detected between treatments for midday leaf water potentials, in June and August 1992 and 1993, clearly pointing out to irrigation effects on LWP patterns. It is possible that the small number of measurements was associated with the absence of more notorious differences. A positive effect was also observed for N, since the highest values of LWP were observed for the more fertilized trees (Fig. 2).

In order to evaluate the driving forces in the soil-plant-atmosphere continuum, a set of regressions were assessed between LWP (predawn and midday), atmospheric parameters and soil volumetric water content (VWC). The results of coefficient of determination (r^2)

are presented in Table 2. Although r^2 values are statistically significant they are lower than midday LWP, suggesting that predawn LWP followed a pattern which may eventually be independent of the environmental conditions. Although it is known that predawn LWP is related to soil water availability this fact was not entirely sustained by the regression values obtained for predawn LWP and VWC. Nevertheless, when we consider "non-irrigated" treatments, which were submitted to soil drought during summer, we obtained a slightly significant correlation ($r^2 = 0.71$ and 0.50), which was similar to the treatments with 50% irrigation levels. For "well-irrigated" treatments (100%) no correlation was obtained, which may be related to the heterogeneity of soil water distribution, due to irrigation effects.

Midday LWP values (Fig. 2) were well correlated (Table 2) with atmospheric conditions, especially with maximum temperature and E_{pan} evaporation. The lowest values were recorded during summer (Fig. 2) for all treatments and we may assume that evaporative demand played an important role in LWP patterns. No correlation was obtained between VWC and LWP, except for 1.5/0 treatment. Considering the reasons stated above we may not exclude some effect of VWC on LWP patterns.

Plants growing under well-irrigated conditions (1.5/100 and 4.5/100) exhibited the highest growth increments all over the 3 years, as shown in Figure 3. As it can be seen in Table 3, branch length increment was statistically significant after full establishment of irrigation (July and August) in 91 and 92. A significant and positive N × water interaction was also observed. In the first and second years the effect of

Table 2. Coefficients of determination (r^2) between leaf water potentials and atmospheric parameters: Maximum air temperature (T_{max}), evaporation pan (E_{pan}), minimum air temperature (T_{min}) and volumetric water content (VWC). $N = 15$, except for VWC were $N = 10$. All the coefficients were statistically significant at $p < 0.05$, except those with superscript ns (non significant)

LWP	Atmospheric parameters	1.5/0	1.5/50	1.5/100	4.5/0	4.5/50	4.5/100
Predawn							
	T_{max}	0.45	0.48	0.42	0.38	0.64	0.43
	E_{pan}	0.50	0.55	0.41	0.49	0.75	0.53
	T_{min}	0.45	0.39	0.37	0.26	0.50	0.30 ^{ns}
	VWC	0.71	0.54	0.24 ^{ns}	0.50	0.49	0.06 ^{ns}
Midday							
	T_{max}	0.81	0.73	0.83	0.66	0.82	0.71
	E_{pan}	0.79	0.74	0.78	0.61	0.75	0.67
	T_{min}	0.75	0.68	0.68	0.73	0.78	0.63
	VWC	0.71	0.31 ^{ns}	0.22 ^{ns}	0.22 ^{ns}	0.22 ^{ns}	0.10 ^{ns}

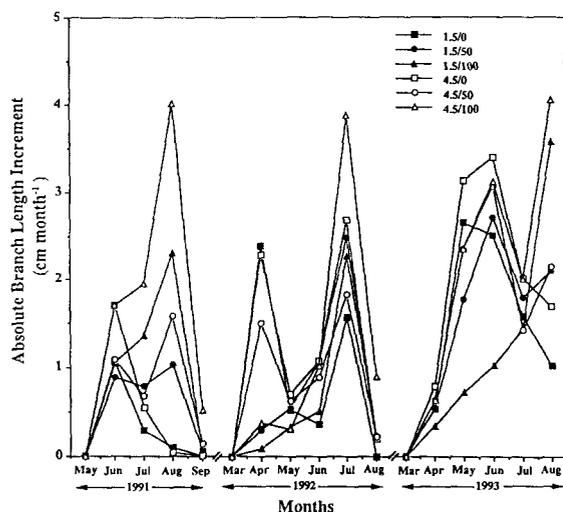


Fig. 3. Absolute branch increment for the fertigation treatments during the 3 years of the experiment. Symbols as Figure 2. Each point is an average of 72 measurements per treatment.

nitrogen and summer irrigation is clear (Fig. 3), since N-high and well-irrigated treatments showed higher growth increments. In 1993 the occurrence of late rainfall in May (Fig. 1) triggered very high initial growth increments (Fig. 3), especially for the non-irrigated treatments.

The increase in fruit yield (Fig. 4) as a response of fertigation is more pronounced in the well-fertigated

Table 3. Significance levels of differences among treatments for branch length increment obtained from multifactorial ANOVA table using F-test. These data are related to Figure 3. Significant at 95% (*) and 99% (**). Non-significative (ns)

Dates	Branch length increment		
	N	Water (W)	N × W
June 91	*	ns	ns
July 91	ns	**	ns
August 91	**	**	**
Sept 91	**	**	**
April 92	**	**	**
May 92	ns	**	ns
June 92	**	ns	*
July 92	**	**	**
Aug 92	**	**	**
April 93	ns	ns	ns
May 93	**	**	ns
June 93	**	**	**
July 93	ns	ns	ns
Aug 93	ns	**	ns

treatment (4.5/100). It should be noted that the increment from 91 to 92 was different between treatments. There was a larger increment from 92 to 93, but similar for all treatments.

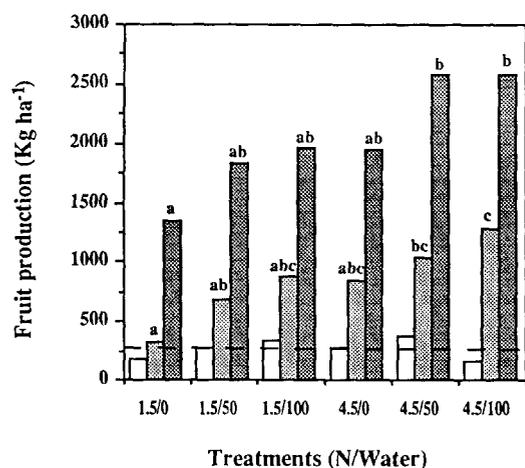


Fig. 4. Fruit production for all treatments. □ May 91; ▨ May 92; ■ 1993. Dashed line is a reference line, representing the mean fruit production for 1991. For each year, values with the same letter are not significantly different at $p \leq 0.05$. The values of the two years were not significantly different at low N levels at $p \leq 0.05$.

Discussion

Contrary to what occurs for several Mediterranean species in natural habitats or in potted plants under greenhouse conditions (Acherar and Rambal, 1992; Correia and Catarino, 1994; Rambal, 1992) predawn values in the dry season for the non-irrigated treatments do not drop below -1.1 MPa, which may indicate a deep-rooting strategy in our conditions. Similar conclusions were suggested by Nunes et al. (1992) also for mature carob-trees. Rhizopoulou and Davies (1991) also found that, in young carob tree plants submitted to soil water deficit for 3 weeks, deep penetration of some roots was enough to supply substantial amounts of water to shoot growth. On the other hand, the direct response of predawn LWP values to irrigation in 1991 (Fig. 2), associated with the increment of branch length increments exhibited by the well-irrigated trees (Fig. 3), may also indicate an important root development or good infiltration in upper soil layers. The same kind of root adaptation was observed in olive trees submitted to drip-irrigation, and grown under dry-farming conditions for several years (Fernandez et al., 1992). The high values of LWP presented during early summer 1993 (Fig. 2), responsible for the high growth increments observed in this period (Fig. 3) can be explained by accumulated rainfall during the previous spring (Fig. 1), recharging soil water and reducing the severe summer drought stress.

The low minimum LWP values registered in well-irrigated trees, showing no clear differences compared with the non-irrigated trees (Fig. 2), was also observed in previous works using drip-irrigated carob-trees (Correia and Martins-Loução, 1990). Water effects were only observed at the end of irrigation season (Table 1). Similarly, Gower et al. (1992) reported no differences in midmorning xylem water potential comparing irrigated and non-irrigated *Pseudotsuga* trees and Torrecillas et al. (1989) also found no differences in minimum LWP values for almond trees submitted to different irrigation regimes. The low values of LWP observed in this study (Fig. 2) may indicate that not enough water had reached the active roots, due to high air evaporative conditions (Fig. 1). However, significant differences were observed for vegetative growth (Fig. 3) showing a significant effect of N, water and $N \times$ water interactions (Table 3). Similarly, reproductive output (Correia and Martins-Loução, 1992, 1993a) and fruit production (Fig. 4) show a positive effect of fertigation treatments. Lloveras and Tous (1992) also observed a significant increase in carob production after nitrogen application in an old carob tree stand and other experiments registered a positive irrigation effect on fruit production (Esbenshade and Wilson, 1986).

Concerning the "N-effect", the hypothesis was that the cumulative effect of nitrogen fertilization would affect foliar area and consequently whole tree water balance, as it was simulated by Rambal (1993). Although our results do not indicate a clear "N-effect", we might expect that the increase in branch growth (Fig. 3) triggered modifications in tree water balance. Under drought conditions, such as the case of the non-irrigated treatments, and in the presence of high levels of N, the increase in N availability, influences carob productivity (Fig. 4) leading to higher output of leaves (Correia and Martins-Loução, 1992) and reproductive organs (Correia and Martins-Loução, 1993a). Considering fruit production per unit of available water it is expected that non-irrigated trees would present higher rates of water use efficiency (Correia and Martins-Loução, 1993b). Although soil drying will eventually reduce root growth (Rhizopoulou and Davies, 1991), it is also established that N accumulation is able to function not only as a N source but also as an osmoticum (Leidi and Lips, 1990). This is in agreement with recent work which shows that osmotic potential can control carob drought stress tolerance (Nunes et al., 1989) and could explain the low values of midday LWP in non-irrigated trees during the summer drought period

(Fig. 2). With an increasing water supply a direct N-mobilization contributed to a higher growth increment (Fig. 3). The increment of leaf and, consequently, of canopy area, forces the plant to change water distribution, explaining the lower LWP values observed (Fig. 2) and also non-significant differences in $N \times$ water effects (Table 1).

Another driving force may also be present which modifies minimum LWP patterns. The regressions obtained between atmospheric conditions and midday LWP (Table 2) showed that the effect of air vapor pressure deficit is more important than the effect of soil water status for the maintenance of the soil-plant-atmosphere continuum, which is in agreement with other studies (Oliveira et al., 1992). On the contrary predawn LWP seems to have some relations with VWC. The absence of correlation for the highest irrigated treatments may be explained by the association between high soil water status and high predawn LWP. However we must admit that probably the sampling for the VWC might not have been sufficiently representative of the soil water status. The large amount of gravels and the rapidly drying soil, especially during summer, made homogeneous soil sampling very difficult. So, the correlations between LWP (predawn and midday) with VWC did not seem to be an appropriate approach to the analysis of soil-plant-atmosphere continuum in this orchard.

Air evaporative demand (Fig. 1) and branch growth (Fig. 3) increase soil-to-leaf water flux which, in turns, condition LWP patterns maintaining the trees under a moderate drought stress, independently of the treatments (Fig. 2). Therefore, this moderate water stress should be regarded as a "functional" stress which is a natural consequence of tree development.

The results presented here are in agreement with the "water-spending" strategy hypothesis suggested by Lo Gullo and Salleo (1988), as a drought adaptation mechanism of carob-tree. Carob-tree can maintain stomatal opening and high leaf water content even with low soil water availability (Nunes et al., 1992), which is allowed by sharply dropping LWP in response to small water losses (Lo Gullo and Salleo, 1988).

It is important to note that the major differences in LWP patterns were observed between non-irrigated (0% E_{pan}) and irrigated treatments (50 and 100% E_{pan}). It means that under these conditions 50% E_{pan} was enough to ensure a reasonable vegetative growth (Fig. 3), reproductive output (Correia and Martins-Loução, 1993) and fruit production (Fig. 4). Similarly, these data also suggest that under the normal conditions of

this type of Mediterranean vegetation, nitrogen application is effective even without irrigation. These results have important implications for practical and economical purposes and thus must be taken into consideration during the establishment of irrigation schedules, especially when semi-arid Mediterranean climates are involved.

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