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Phloem Translocation and Heat-induced Callose Formation in Field-grown *Gossypium hirsutum* L.[1]

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ABSTRACT

Phloem translocation rates in field-grown cotton (*Gossypium hirsutum* L.) dropped from morning to afternoon and continued to decline toward evening, except that recovery occurred following the hottest afternoon when the maximum temperature was 44 C. Water deficits increased from morning to evening, and severity of deficits generally were proportional to daytime heating. Water stress contributed toward reducing translocation but was not always the governing factor. Callose breakdown appeared to be slower than heat-induced synthesis, and in the evening callose still reflected the influence of high afternoon temperatures. Translocation was considerably reduced when about 50% or more of the hypocotyl sieve plates had large amounts of callose. While heat-induced callose may have reduced translocation because of sieve plate pore constriction, temperatures of 39 to 44 C appeared to inhibit an additional component of translocation as well, possibly in the leaf blade.

Laboratory experiments performed on plants grown under artificial conditions have indicated that efficiency of phloem translocation is much reduced at high temperatures ordinarily encountered during the summer (7). Whittle (10) speculated that plants growing in climates in which temperatures frequently rise above 30 C would have either a higher optimum for phloem transport or else survive with a less efficient system. Neither alternative may be necessarily true, however, since above normal rates of basipetal phloem transport may occur some time after the high temperature treatment (3). The conducting system may still be efficient if high rates of flow can take place at night following exposure to daytime temperatures in excess of 30 C. The conductive capacity of phloem may considerably exceed those obtained under normal rates of translocation (8).

Amounts of callose increased in experimentally heated tissues (*cf.* 8), and this buildup was correlated with inhibition of both basipetal (3) and lateral (9) phloem translocation. Basipetal translocation was inhibited by heating 4 cm of hypocotyl; a 1-cm treatment was not effective (3). Increased resistance due to constriction of plasmodesmata and sieve plate pores by callose appeared to be responsible for the inhibiting effect of heating. Electron micrographs (5) showed almost complete constriction of pores by heat-induced callose in intact cotton stems.

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Maximum callose depositions were formed in greenhouse-grown plants following temperature treatments as low as 40 C for 15 min (3). Heat-induced callose formation in phloem was reversible, however. Depositions on sieve plates decreased within 6 hr after heating and were reduced to virtually normal levels in 2 days. Basipetal phloem translocation continued to be inhibited for at least 3 hr following heating; after 6 hr, rates were equal to or above normal (3). There are no reports of

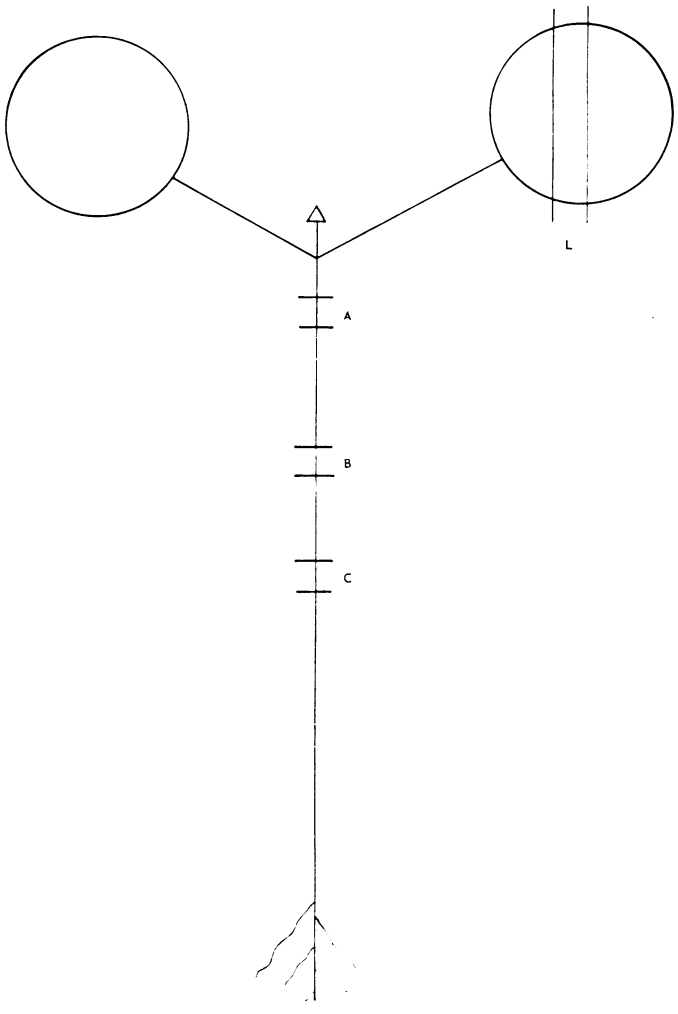

FIG. 1. Diagrammatic localization of samples that were examined for callose and radioactivity. A, B, and C are 4.3 mm long hypocotyl segments cut 0.5 cm, 2.5 cm, and 4.0 cm, respectively, from the cotyledonary node. L is a 4.3 mm wide leaf blade strip cut at an angle of 45° to the midrib.

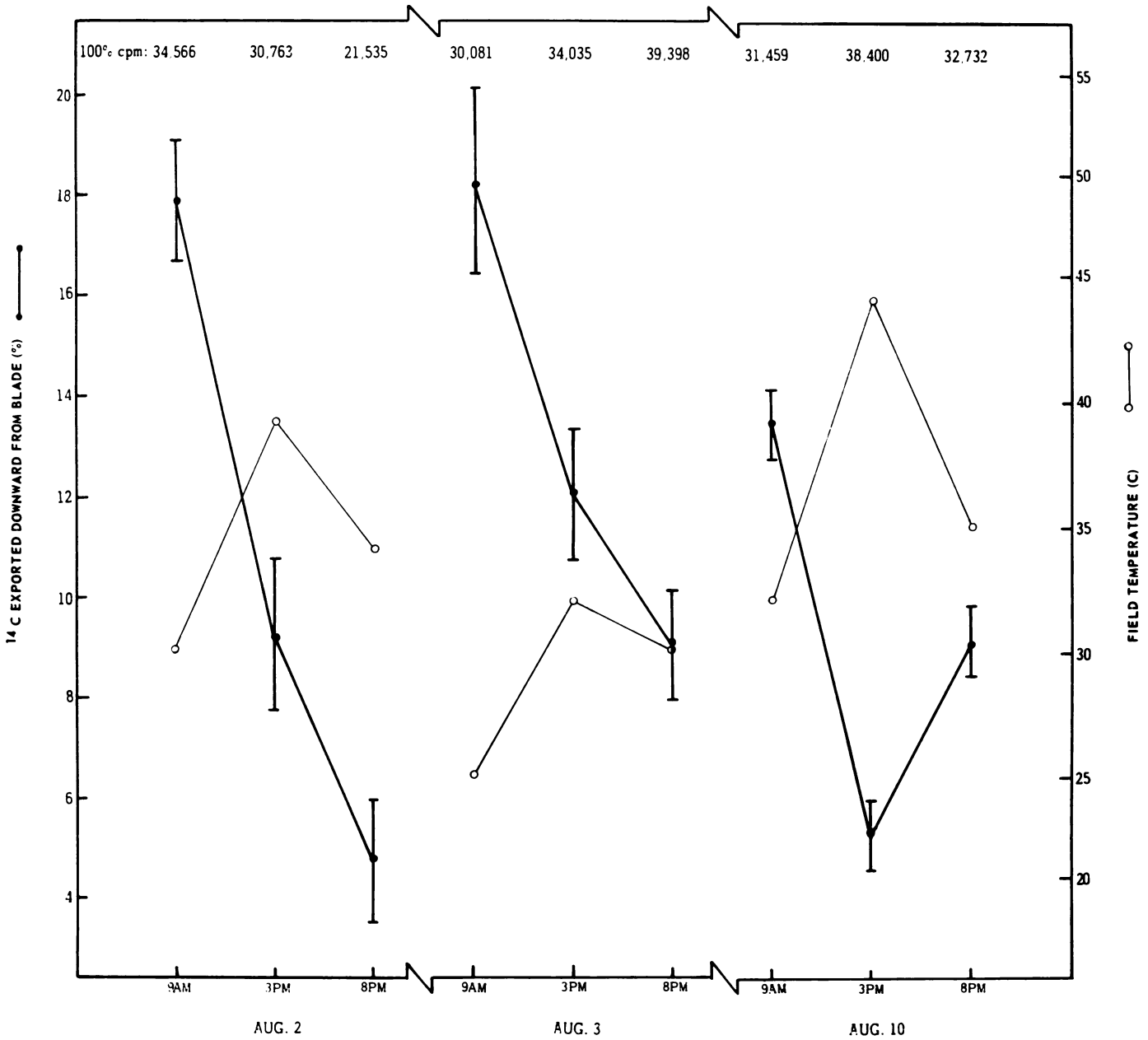

FIG. 2. Basipetal ^{14}C-phloem translocation in field-grown cotton plants brought into the laboratory on 3 days. Each datum of per cent translocation and 100°c cpm is the mean for six plants. Field temperatures were measured 10 cm above the shaded soil surface at time of plant removal.

stimulation of callose formation by natural heating in the field.

An important environmental variable encountered in field work is water stress, which markedly affects phloem translocation (*cf.* 2). Increased water stress reduced both $^{14}CO_2$ uptake by leaves and the amount of radioactivity translocated from them in yellow poplar seedlings. Translocation was reduced to the same extent as $^{14}CO_2$ uptake; stomatal closure was considered to be the principal causative factor (4). High water deficits in cotton (McNairn, unpublished data) and wilting in tobacco (1) did not increase callose in sieve plate pores.

In view of the foregoing, summer field experiments were conducted to study phloem translocation as a function of temperature, natural callose formation, and water stress.

MATERIALS AND METHODS

Gossypium hirsutum L., cv. Acala 4-42 and the newer Acala SJ-1 were initially tested for responses of callose formation and translocation to heat. No appreciable differences between the two varieties were noted, and further work was restricted to Acala SJ-1.

Samples for ^{14}C-translocation, water deficit, and callose levels were taken from 2- to 4-week-old plants in the morning, afternoon, and evening during two summers in Chico, California. Sampling times indicated were Pacific Daylight Time and were within 15 min of solar time. Wind screens were erected to eliminate wind as a potential mechanical stimulant of callose formation. Appropriate insecticides were applied as required and checked for possible influence on callose formation. Treflan (Elanco Co.) was applied prior to planting for weed control. Soil fertility was adequate for short growth periods, and no fertilizer was applied. Sprinkler irrigation was timed so that the soil surface would be dry by afternoon.

Tests of ^{14}C-translocation were all undertaken in the labora-

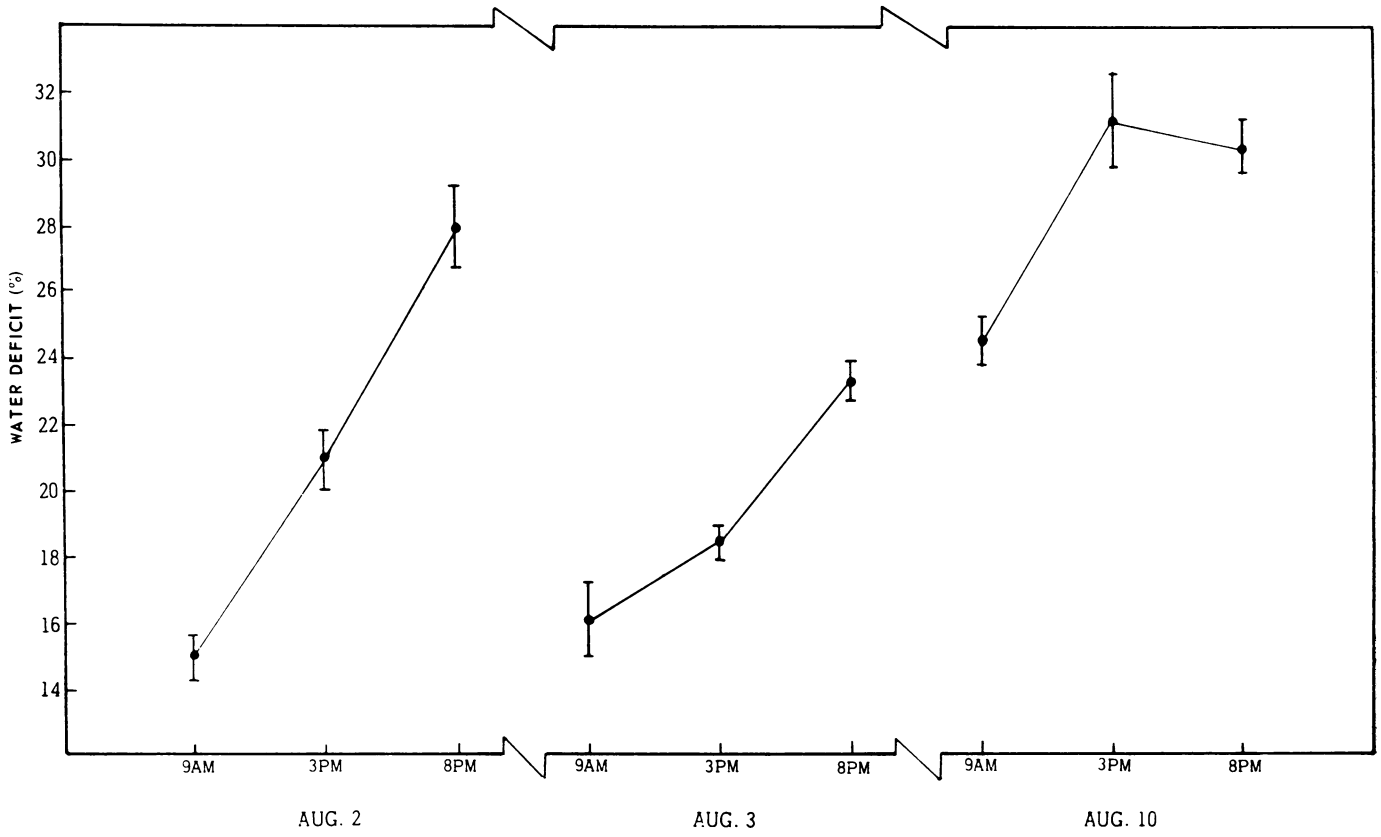

FIG. 3. Leaf water deficits of cotton plants at time of removal from field. Each datum is the mean for six plants. Brackets represent the standrd errors of the means.

tory and were modifications of those previously used (3). Field plants were carefully transferred to containers with soil and roots intact, watered, and kept in plastic bags during transport to the laboratory. Within 45 min, six plants were simultaneously exposed to $^{14}CO_2$ generated from $Ba^{14}CO_3$ with a specific radioactivity of 10 μc/mg. A sealed Plexiglas chamber was used to expose a single cotyledon of each plant to $^{14}CO_2$ for 5 min at the dosage level of 6 μc per plant, with heat-filtered illumination of 800 ft-c provided. Plants were allowed a translocation period of 1 hr at 26 C.

For counting radioactivity, 4.3 mm long hypocotyl segments (Fig. 1) were cut 0.5 cm (segment A), 2.5 cm (segment B), and 4.0 cm (segment C) from the cotyledonary node with a double-bladed knife. Leaf blade strips 4.3 mm in width were cut at an approximate angle of 45° to the midrib. Samples were heated briefly in a minimum of 80% ethanol, ground, heated once more, and transferred quantitatively to planchets, dried, and counted 5 min with a Geiger-Müller tube. The proportion of counts in leaf blade strips to counts in the whole blade was considered to be the same as the proportion of their respective weights. Percentage of translocation was calculated as the sum of cpm in A, B, and C segments divided by the sum of cpm in A, B, and C and the entire leaf blade. Brackets in Figures 2 and 5 represent standard errors of means.

Callose ratings were obtained via fluorescence microscopy, using the aniline blue method previously described (3). Amounts of callose on hypocotyl sieve plates (segments A, B, and C, Fig. 1) were classified as either "faint" or "bright." Faint included clearly visible plates with a thin, whitish yellow appearance, while bright was used to describe all thickly callosed sieve plates, except those covered with definitive callose in the narrow protophloem elements.

Water deficit in cotyledons was measured by Stocker's method (6) at the time of field sampling for callose, and again following the period of ^{14}C-translocation. Cotyledons opposite those exposed to $^{14}CO_2$ were used in the latter sampling and gave values within ± 2% of those determined in the field. Brackets in Figure 3 represent standard errors of means.

Temperatures of the air 10 cm above the soil surface, of plant surfaces, and of internal hypocotyl tissues were all measured with appropriate Yellow Springs Instrument Co. telethermometer probes. Internal measurements were taken from the approximate center of the hypocotyl. In the afternoon, unshaded plant surfaces were approximately 4 C higher than air temperature. Above ground internal hypocotyl temperatures were 2 to 5 C below, and at the soil surface up to 7 C above, air temperature. Cotyledons provided essentially no shading to hypocotyls.

RESULTS

Translocation. Figure 2 illustrates basipetal phloem translocation on 3 days in field plants brought into the laboratory. The afternoon of August 2 warmed to 39 C; August 3 was relatively cool, while August 10 was exceptionally hot, reaching 44 C in the afternoon. On August 3, plants had higher rates of translocation for most of the day than did those on the warmer day of August 2. The lowest afternoon translocation rate occurred on the hottest day, August 10. On all days translocation was maximum in the morning and dropped in the afternoon. It continued to drop in the evening with the exception of the recovery following the hot afternoon of August 10. The evening declines indicated that translocation was being influenced by one or more factors other than temperature at time of sampling.

Water Deficit. Figure 3 illustrates the course of plant water

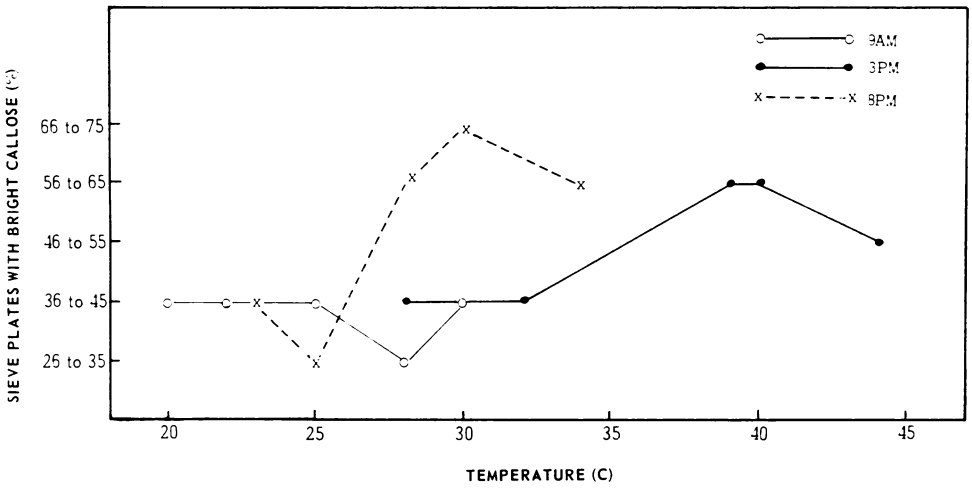

FIG. 4. Callose ratings of hypocotyl sieve plates during morning, afternoon, and evening on various days over the course of two summers. Each datum is the mean for five plants and at least 100 sieve plates per plant.

deficit for the days of August 2, 3, and 10. Each day water deficits increased from morning to evening. Magnitude of deficits generally depended on daytime heating. August 3, the coolest day, caused the lowest deficits, whereas August 10 was hottest and caused the greatest water stress.

There was an inverse relationship between water deficit and amounts of translocation, with one exception. On August 2 and 3 translocation declined with an increasing water stress. August 3 was cooler, caused a lower water stress, and permitted higher rates of translocation. On August 10, however, despite a continued severe water deficit, translocation recovered considerably in the evening. It appears that in this instance a drop in air temperature to 35 C from the excessively high level of 44 C influenced the recovery of translocation.

Callose. Figure 4 indicates the relationship of amounts of callose on sieve plates during morning, afternoon, and evening sampling times to air temperatures on various days over the course of two summers. Callose was always present in minimum amounts during the morning. In the afternoon, amounts were generally higher, especially when temperature was in excess of 35 C. Values for callose in the evening overlapped those for morning and afternoon. The rate of callose breakdown appeared to be slower than its synthesis. At 8 PM callose was still exhibiting the effect of the higher afternoon temperatures, which reached maxima between 3 and 5 PM. By morning callose was at a minimum, regardless of how high the temperature had been on the previous day.

That callose in the evening was higher at 30 C than at 34 C is accounted for by different rates of cooling on these days. It is not so important to distinguish between the callose levels depicted at 39 C and 44 C as it is to note that they are both near or above 50%, at which level axial phloem translocation is considerably reduced.

Figure 5 indicates the relationship of sieve plate callose to phloem translocation. Translocation was considerably reduced when about 50% or more of the sieve plates had high amounts of callose. Even at these higher callose levels, some additional effect of temperature was important. For example, the two low values of 7.2% and 7.0% translocation were measured when field temperatures were 39 C and 44 C, respectively, but callose was not at maximum levels. Higher rates of translocation occurred with callose at the highest levels of 58 to 70%, but temperatures were lower. This would suggest that, while heat-induced callose may have reduced translocation via sieve plate pore constriction, an excessively high temperature affected another component of translocation as well.

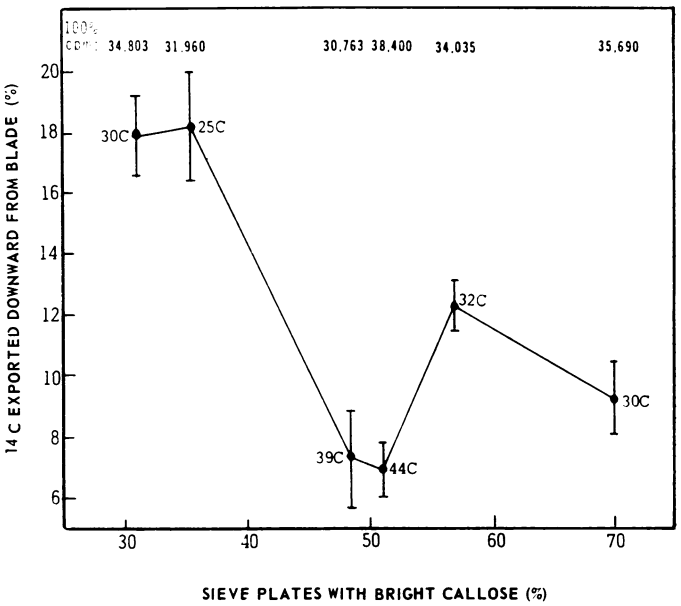

FIG. 5. Relationship of hypocotyl sieve plate callose to basipetal phloem translocation of field-grown cotton with temperature uncontrolled. Each datum of per cent translocation and 100% cpm is the mean for six plants; for callose, the mean for five plants and at least 100 sieve plates per plant. Temperatures were measured at 10 cm above shaded soil surface at time of plant removal. The two lowest callose values were 9 AM samples; the highest callose value, 8 PM; all others, 3 PM.

DISCUSSION

The ^{14}C-translocation tests were conducted at a room temperature of 27 C and utilized plants removed from the field. Thus differences in plant behavior were a consequence of residual environmental effects. Both callose and water deficits were essentially unchanged during removal and testing of plants. Average percentage of water deficit in the laboratory was always within ±2% of the corresponding field value.

Previous work (3) has indicated that sieve plate callose formed when hypocotyl temperatures are high is a factor in blockage of longitudinal phloem translocation. The effect is residual; a 40 to 45 C treatment along 4 cm of hypocotyl for only 15 min is sufficient to bring about callose buildup and inhibition of translocation for several hours. Both callose and

translocation return to near normal levels within 6 hr (3). This phenomenon with respect to translocation occurred on August 10 (Fig. 2). The 44 C afternoon air temperature brought about a considerable drop in axial translocation through the unshaded hypocotyl, but some recovery was noted in the evening when the temperature had dropped to 35 C. Nearly complete inhibition of phloem transport was followed by a surge of phloem transport which was above normal for 35 C.

Significantly, recovery occurred despite a high (30.4%) water deficit. At the same time, callose breakdown in hypocotyls of these field plants did not occur as rapidly as in the greenhouse-grown plants of McNairn and Currier (3), which had been returned to lower temperatures immediately following heating. It appears that a considerable buildup of phloem assimilate in leaves may create enough pressure to partly counteract the inhibition of movement by sieve plate pore constriction. Presumably this high internal phloem pressure could still arise in leaves despite a high over-all water deficit in the plant.

Inhibition of translocation at the highest temperatures was more severe than expected for observed callose buildups. In previous work (3) initial reduction of translocation occurred when about 50% or more of the sieve plates in the hypocotyl exhibited high callose levels. Figure 5, however, depicts greatly reduced translocation at callose levels of 49% and 51%; corresponding air temperatures were 39 C and 44 C, respectively. In these experiments there may be a further inhibitory effect on translocation apart from that caused by the heat-induced callose buildup. The entire shoot system experienced high field temperatures, while in the previous laboratory work only a portion of the hypocotyl was heated (3). High temperatures may bring about separate responses in different components of phloem translocation. For example, lateral movement of radioactive phloem assimilate does not recover as quickly from heating as does axial movement (3, 8). Heat in the leaf blade would affect at least one phloem mechanism, vein loading, which is of a different nature than basipetal flow in the hypocotyl. Vein loading, largely an enzymatic process, could be adversely affected by heat to a greater extent than axial pressure flow in the hypocotyl.

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