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Diurnal Water Balance of the Cowpea Fruit[1]

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ABSTRACT

The vascular network of the cowpea (*Vigna unguiculata* [L.] Walp.) fruit exhibits the anatomical potential for reversible xylem flow between seeds, pod, and parent plant. Feeding of cut shoots with the apoplast marker acid fuchsin showed that fruits imported regularly via xylem at night, less frequently in early morning, and only rarely in the afternoon. The dye never entered seeds or inner dorsal pod strands connecting directly to seeds. Root feeding (early morning) of intact plants with $^{32}PO_4$ or $^{3}H_2O$ rapidly (20 min) labeled pod walls but not seeds, consistent with uptake through xylem. Weak subsequent (4 hours) labeling of seeds suggested slow secondary exchange of label with the phloem stream to the fruit. Vein flap feeding of subtending leaves with [^{14}C]sucrose, $^{3}H_2O$, and $^{32}PO_4$ labeled pod and seed intensely, indicating mass flow in phloem to the fruit. Over 90% of the ^{14}C and ^{3}H of fruit cryopuncture phloem sap was as sucrose and water, respectively. Specific ^{3}H activities of transpired water collected from fruits and peduncles were assayed over 4 days after feeding $^{3}H_2O$ to roots, via leaf flaps, or directly to fruits. The data indicated that fruits transpired relatively less xylem-derived (apoplastic) water than did peduncles, that fruit and peduncle relied more heavily on phloem-derived (symplastic) water for transpiration in the day than at night, and that water diffusing back from the fruit was utilized in peduncle transpiration, especially during the day. The data collectively support the hypothesis of a diurnally reversing xylem flow between developing fruit and plant.

The common observation that rapidly growing fruits lay down dry matter much faster than they fix carbon photosynthetically or attract solutes from dilute xylem fluids by transpiration, has led to the general conclusion that fruits and seeds are nourished primarily by intake through phloem (1, 2, 6, 8, 15). Knowing the dry matter content of phloem sap entering a fruit, and assuming that such import is by mass flow, it has been estimated that in certain species (*e.g.* Yucca and palms [16] and the legumes lupin [*Lupinus albus*] [11] and soybean [*Glycine max*] [4]) fruits acquire just sufficient water through phloem for tissue growth, so that any additional water required for transpiration is likely to be met by xylem import. In another group of species, however (squash [*Cucurbita pepo*] [17], bean [*Phaseolus*] [5], and sausage tree [*Kigelia*] [3]), phloem has been suggested to supply water in excess of the fruit's requirements in growth and transpiration, a situation assumed to result in positive back flow of water to the plant, probably via the xylem. This paper reports on tracer studies of the diurnal exchanges of solutes and water by fruits of cowpea (*Vigna unguiculata*), a species whose fruits are also suspected to generate surplus water through phloem import (12). The data obtained are related to the fruit's anatomy and to transpiration studies of fruit, inflorescence stalk, and whole plant.

MATERIALS AND METHODS

Plant Material. Nodulated plants of cowpea (*Vigna unguiculata* [L.] Walp cv Vita 3:*Rhizobium* CB756) were grown in sand culture in a naturally lit, temperature-controlled glasshouse during winter (May–July) or summer (December–February). Temperatures ranged from 28 to 32°C day, 20 to 22°C night in winter, 32 to 38°C day, 23 to 26°C night during summer. Plants were watered twice daily and received N-free mineral nutrient solution throughout growth (9). Fruit age was determined by tagging flowers at anthesis, and all experiments were conducted on first formed fruit (see 9).

Transpiration Measurements on Whole Plants. Attached Fruits, and Peduncles. Whole plant transpiration was measured gravimetrically on a day:night basis from anthesis onwards during summer, using potted plants whose root systems were sealed within plastic bags to restrict weight losses solely to plant transpiration.

Fruits and peduncles (inflorescence stalks) were enclosed separately into glass cuvettes through which air was passed at a rate of 2 L/min. A water screen above the plants restricted temperature fluctuations within the cuvettes to within 4°C of ambient. Transpiration rates were determined by measuring changes in water vapor content of the entering and outgoing gas streams using a humidity probe (Vaisala humicap, Helsinki, Finland) (12). Where $^{3}H_2O$ labeling studies were involved, the transpired water of fruit or peduncle was collected into freezer traps attached to the effluent streams of the cuvettes. The water was then weighed and assayed for ^{3}H.

Anatomical Investigations of the Vasculature of the Fruit. The vascular network of pod and seeds was examined using fruits which had been dehydrated in ethanol and then cleared in methyl benzoate.

Vascular tissues of specific regions of the fruit were studied using glutaraldehyde-fixed material embedded in Spurr's resin (13). Transverse sections (2 μm) were examined by light microscopy following staining with toluidine blue.

Labeling Experiments on Water and Solute Exchange between Fruits and Parent Plant. Feeding of $^{3}H_2O$ involved application to the rooting medium of intact plants (Table I, 1a), through the cut base of shoots (Table I, 2a), to whole plants through the xylem via a mid-vein flap on the central leaflet of the leaf subtending the first formed fruit (Table I, 3b; and for further details of technique, see Ref. 10), or directly to an attached fruit by surface application or injection into the upper distal wall of the pod (Table I, item 4). Assays for ^{3}H were conducted on tissue extracts of fruit and peduncle, cryopuncture phloem sap of fruits (see 10), and transpired water collected from peduncles and fruits.

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Table I. *Labeling Experiments Used in Study of the Exchange of Water and Solutes in Xylem (Apoplast) and Phloem (Symplast) between Fruits and Parent Plant of Cowpea*

Method of Application	Tracers Used	Dose/Plant	Duration of Experiment	Harvests, Sap Collections, and Tissues Assays Undertaken
1. To rooting medium of intact plant	(a) ^3H_2O	200 μCi	2 d	^3H assays of peduncles, seeds, and pod walls. ^3H assay of transpired water of peduncle, fruit, and nurse leaf.
	(b) $^{32}PO_4$	50 μCi	2 h	Time course of labeling of seeds and pod tissues (20–100 min after application).
2. To cut shoot through transpiration stream	(a) ^3H_2O or [^{3}H]inulin	100 μCi	4 h	Time course studies of ^3H labeling of shoot and fruits.
	(b) Acid fuchsin	1% w/v	1 h	Day and night feedings and study of distribution of dye in shoot and fruit parts by visual examination and sectioning of plants.
3. To mid-vein flap on nurse leaf through transpiration stream	(a) Acid fuchsin	1% w/v	2 h	Visual inspection of fed leaf and other plant organs for dye uptake
	(b) ^3H_2O	200 μCi	4 d	^3H assays of peduncles, seeds, and pod walls. ^3H assay of transpired water of peduncle and fruit. ^3H assay of fruit (cryopuncture) phloem sap.
	(c) [^{14}C]sucrose	20 μCi	5 h	Time course of ^{14}C translocation to and accumulation in peduncle, seeds, and parts of pod. ^{14}C assay of fruit (cryopuncture) phloem sap.
	(d) $^{32}PO_4$	20 μCi	3 h	Time course of ^{32}P labeling of seeds and pod tissues.
	(e) [^{3}H]inulin	100 μCi	4 h	Assay for ^3H in parts of plant other than fed leaf.
4. Surface application or injection into attached fruit	$[^3H]_2O$	50 μCi	4 d	Time course of ^3H distribution in peduncle and shoot parts. ^3H assay of transpired water of peduncle and nurse leaf.
5. To cut distal tip of attached fruit	Acid fuchsin	1% w/v	6 h	Study of dye distribution by visual examination and sectioning of plant parts.

Application of [U-^{14}C]sucrose via leaf flaps (Table I, 3c) provided a time course of phloem translocation from leaf to pod and seeds. Feeding of [^{3}H]inulin, through a leaf flap (Table I, 3e) or to a cut transpiring shoot (Table I, 2a) was used to identify apoplastic uptake by fruits from leaf or stem (see 14).

$^{32}PO_4$ applications via the rooting medium (Table I, 1b) or a leaf flap (Table I, 3d) were employed to study the transfer of phosphorus to seed and pod via apoplast or symplast, respectively.

Transpired water was assayed for ^3H, and ethanol-soluble tissue extracts assayed for ^{32}P, ^{14}C, or ^3H using a Beckman LS7500 scintillation counter, with counting programs corrected for quenching and fluorescence.

Feeding of the Apoplast-Mobile Dye Acid Fuchsin. Fruiting shoots were cut at the base under water and transferred for 1 h to acid fuchsin solution (1% w/v) (Table I, item 3a). Red coloration of interveinal mesophyll of leaves or subepidermal tissues of fruit, fruit stalk, and stem gave evidence of xylem (apoplastic) uptake by an organ. Xylem strands distributing the dye were identified by hand sectioning.

Dye feedings were conducted at a range of times of the day and night under ambient glasshouse conditions in winter and summer, using at least 100 plants in each season with first-formed fruits aged from 6 to 22 d after anthesis.

Acid fuchsin applied via the cut distal tips of attached fruits (Table I, item 5) tested whether an apoplastic (xylem) pathway existed for back flow of water and solutes between fruit and parent plant.

RESULTS AND DISCUSSION

Transpiration of the Whole Plant, Fruits, and Peduncles (Figs. 1 and 2). Plants transpired from 25 to 134 ml per plant during a day, and 2 to 7 ml during a night (Fig. 1A). A total of 1830 ml water were transpired and 6.5 g dry matter accumulated over post-anthesis development, giving a transpiration ratio of 282 ml/g.

Fruit transpiration was higher during the day than at night, but the differential was much less than for the whole plant (*cf.* Fig. 1, A and B). The daily rate of water loss increased with increasing fruit size for the first 9 d, remained reasonably constant until 19 d, and then increased suddenly as the membrane integrity of the fruit deteriorated during pod senescence and fruit dehydration. All fruits on the plant showed similar transpirational patterns and the combined losses of a plant's fruits represented some 3 to 8% of its total water utilization during reproductive development.

Both the peduncle and its subtended fruit exhibited diurnal fluctuations in transpiration under ambient glasshouse conditions, with maximum rates of water loss tending to occur in the early afternoon (*e.g.* data shown in Fig. 2). However, a fully sized fruit lost water at a lesser rate in the day than did its peduncle, while at night transpiration rates of the two organs were nearly

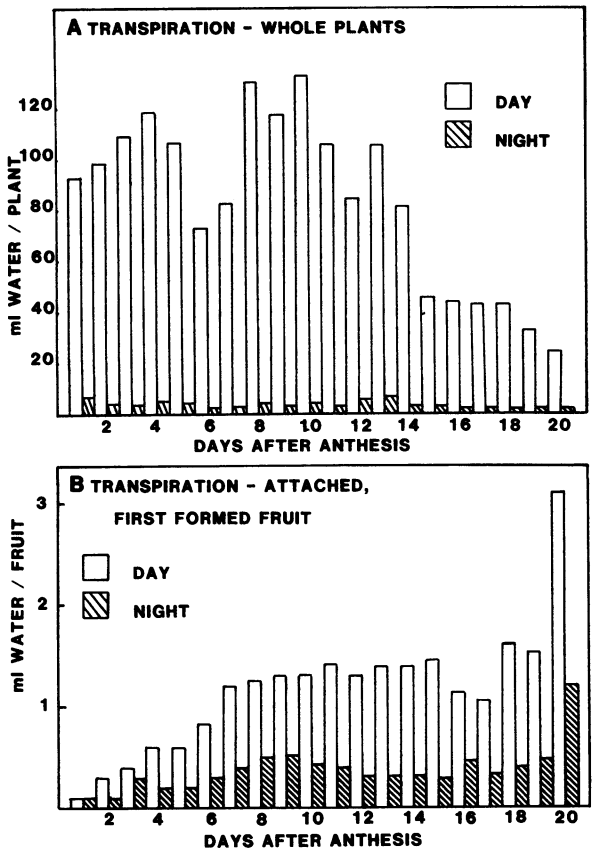


FIG. 1. Post-anthesis water economy of whole plants (A) and attached first-formed fruits (B) of symbiotically dependent cowpea (*V. unguiculata* cv Vita 3). Each day and night value for transpiration rate is a mean value for 15 plants (A) or six fruits (B). Note greater day:night differential in transpiration of whole plant than of fruit.

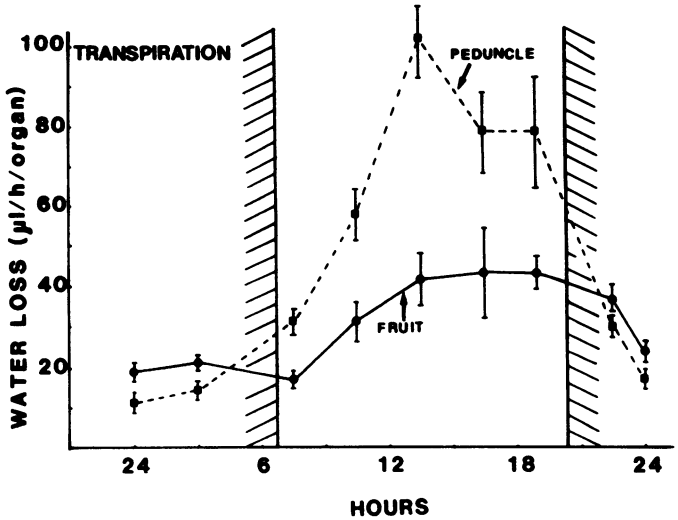


FIG. 2. Diurnal changes in transpiration loss of fruit and peduncle of summer-grown cowpea (*V. unguiculata*). The data points and their SE represent the means of sequential measurements from the same set of 10 plants.

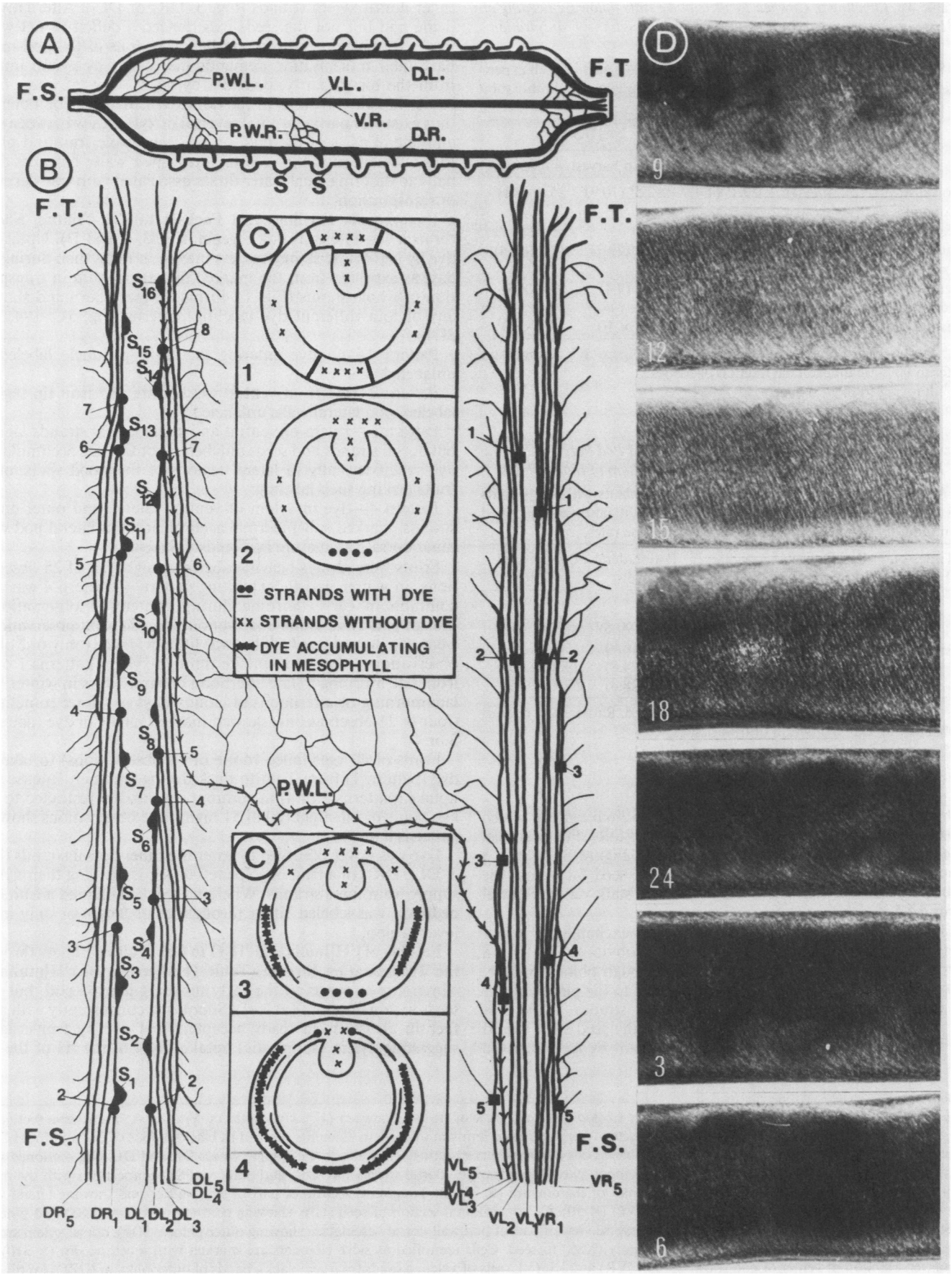
equal. Daytime water losses per unit area were five to seven times less in fruits than in peduncles.

Vasculature of the Fruit (Figs. 3 and 4). The xylem supply to the venter of the pod comprises left hand (Figs. 3A and 4E, VL; Fig. 3B, VL_1–VL_5) and right-hand (VR; (VR_1–VR_5) longitudinal strands of xylem elements, originating from vascular tissues of the fruit stalk (FS). Each xylem strand bifurcates (Fig. 3B, numbered black squares) in its upward passage through the fruit, the outermost strands branching near the fruit base, the others at progressively higher levels. Each pair of daughter xylem branches run together for a distance up the fruit before the outermost member moves out to join the vein network of the pod wall (Fig. 3B, PWL). Branching of xylem strands roughly compensates for lateral losses of daughter strands to the pod wall, so that some 8 to 10 groups of xylem elements are regularly visible in cross-sections (*e.g.* see Fig. 4E) of any but the tip region of the ventral vasculature. The xylem network of the dorsum of the pod (Fig. 3A, DL; Fig. 4A, DR) also comprises left hand (DL_1–DL_5) and right hand (DR_1–DR_5) sets of xylem strands (see Fig. 3B). The outer four members (DL_2–DL_5 and DR_2–DR_5) of each set fuse in turn (Fig. 3B, numbered circles) with the corresponding innermost strand (DL_1 or DR_1) serving the seeds (Fig. 3A, S; Fig. 3B, S 1–16) attached to left or right halves of the pod. Some dorsal and ventral strands end blindly in the fruit tip (FT) (see Fig. 3, A and B).

Conventional external phloem (Fig. 4, OP, OPV, OPD) accompanies all parts of the xylem network described above. Each seed receives a direct and continuous xylem (XSS) and phloem supply (PSS) from the dorsal suture via the seed stalk (SS) (Figs. 3, A and C). Internal strands consisting only of phloem (Fig. 4A and 4D, IP) connect outer and inner lateral components of the dorsal vasculature.

The xylem of the vasculature of the fruit thus comprises a fully integrated system, with cross-connections between dorsum and venter via the lateral pod vein network and limited communication between strands of the longitudinal network by fusions (dorsum) or branching (venter) of xylem strands. Thus, in the fruit illustrated in Figure 3B, all seeds connect directly with the xylem of the fruit stalk, via DL_1 or DR_1, while individual seeds have their own close connections with specific regions of the pod

FIG. 3. A, Diagrammatic representation of the vascular network of a fruit of cowpea (*V. unguiculata* cv Vita 3), depicted as if split along its dorsal suture and opened out to show where the seeds (S) are attached to the left (DL) and right (DR) halves of the dorsal vasculature. Parts of the lateral pod wall vein network (PWL, PWR) are indicated. (F.S.), fruit stalk; (F.T.), fruit tip; (VL and VR), left and right halves of the ventral vasculature. B, Detail of vein network of dorsum (DR_1–DR_5; DL_1–DL_5), venter (VR_1–VR_5; VL_1–VL_5) and part of lateral wall (PWL), showing branching of ventral longitudinal strands (numbered squares), fusions of longitudinal dorsal strands (numbered circles), and points of attachment of seeds (S_1–S_{16}) to the dorsal vasculature. Part of the lateral vein network (PWL) of the pod wall is indicated. The pathway marked by arrows traces a continuous xylem connection between the distal seeds (S_{15} and S_{16}) via the pod wall lateral vasculature (PWL) to the ventral longitudinal strand VL_4. C, Patterns of labeling of fruits following transpirational feeding of cut shoots with the apoplast marker acid fuchsin. Dye distribution in transverse section of the fruit is shown pictorially as presence or absence of dye in xylem of ventral, lateral, and dorsal parts of the pod. Note that the seed stalk and inner dorsal pod strands always fail to accept the dye. (The pod section C_1 is partitioned into sectors indicating how harvested pods were cut up longitudinally for tissue radioassays in the isotope-labeling studies) (Tables III–V). D, Photographs of the mid lateral surfaces of fruits following 1 h of feeding of transpiring shoots with acid fuchsin at a range of times over a diurnal cycle (winter conditions). Dark regions on the photographs denote uptake of the red dye. Note heavy labeling (xylem uptake) of fruits at night (2400–0600 h), lesser labeling in early morning (0900 h) or early evening (1800 h), and no uptake of dye over the period 1200 to 1500 h (magnification, × 2.5).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C
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STRANDS WITHOUT DYE
DYE ACCUMULATING IN MESOPHYLL
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3
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VL5 VL4 VL3 VL2 VL1 VR1 VR5
D
9
12
15
18
24
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6

Table II. *Labeling of Cowpea Leaflets and Fruits following Feeding of ^3H_2O or [^{3}H]Inulin to Cut Fruiting Shoots through the Transpiration Stream*

See Table I for details of feeding. Two replicate plants in each experiment. Fruits 16 to 18 d, winter grown, and fed under full illumination in the morning (10 h).

	[^{3}H]Inulin fed		^3H_2O fed	
	20 min harvest	4 h harvest	20 min harvest	4 h harvest
Dorsal strip of pod[a]	22.4[b] (5.3)[c]	29.1[b] (4.4)[c]	18.5[b] (10.9)[c]	25.4[b] (9.1)[c]
Seeds	0 (0)	0 (0)	0 (0)	6.9 (34.2)
Lateral pod walls	6.8 (90.0)	29.1 (92.0)	2.2 (79.7)	11.7 (48.4)
Ventral strip of pod	26.2 (4.7)	19.7 (3.6)	7.6 (9.4)	20.4 (8.3)
Leaflets	121×10^3	141×10^3	69×10^3	49×10^3

[a] Tissues of fruits dissected as indicated in Figure 3C. Radioassays refer to 80% ethanol-soluble fraction of tissues.
[b] dpm/mg fresh wt tissue.
[c] % of total label of fruit, in parentheses.

Table III. *Time Course of Labeling of Seeds and Pod Parts of Cowpea Fruits after Feeding $^{32}PO_4$ to the Rooting Medium of Intact Plants*

All data are for single experiments on single plants with study fruits 15 to 17 d old. Winter grown plants were fed at noon and supplied continuously with the isotope. See Table I for dose rates.

Times of Harvest	Pod Dorsal[a] Strip	Seeds	Pod Lateral Walls	Pod Ventral Strip
h				
0.5	1.1[b] (3.8)[c]	0.1[b] (3.2)[c]	1.6[b] (90.6)[c]	0.7[b] (2.4)[c]
1.0	258 (16.3)	36 (39.3)	22 (16.9)	62 (27.6)
1.5	1020 (15.1)	310 (36.5)	65 (24.8)	1920 (23.6)
2	2790 (12.7)	370 (39.4)	370 (28.4)	5700 (19.5)

[a] See Figure 3C for regions of the fruit harvested. Radioassays refer to 80% ethanol-soluble fraction of fruit tissues.
[b] dpm/mg fresh wt tissue.
[c] % of total label of fruits, in parentheses.

lateral vein network (PWL) via any longitudinal strands which fuse in their immediate vicinity with DL_1 or DR_1. For example, the cross-connection marked by arrows in Figure 3, defines a continuous xylem pathway from the distal seed S_{16} across the dorsal and lateral vein networks to the seed stalk via the ventral strand VL_4.

The implications of the above to xylem exchanges of water between pod, seed, and peduncle should be obvious. If both pod and seeds generated an excess of water through phloem intake, excess water from the pod would drain back to the plant via any of the ventral or outer dorsal longitudinal strands, while that exported from seeds through the xylem of the seed stalk would be likely to return directly to the parent plant by means of the inner dorsal xylem strands (Fig. 3, DR_1 or DL_1). Alternatively, if the pod but not the seeds experienced a deficit in net water intake by phloem, ventral and lateral regions of the pod might have their transpiration demands met partly by xylem import from the plant, partly, possibly, by lateral flow of water from seeds via xylem fusions in the dorsal vasculature. The potential thus exists for partial or total reversal of xylem flow between seed and lateral parts of the pod, or between whole fruit and plant, thus allowing fruit and seed to adjust their water balances diurnally to differing local water fluxes associated with translocation or transpiration.

Experiments Feeding Acid Fuchsin to Cut Fruiting Shoots through the Transpiration Stream (Fig. 3, C and D). Uptake of dye by leaves and stems was less intense at night than during the day, as expected from the marked diurnal rhythm in transpiration of whole shoots (Fig. 1). Fruits on the other hand showed any of four different dye labeling patterns (Fig. 3C, 1–4, Fig. 3D).

Pattern 1—no dye entering the fruit; peduncle labeled or unlabeled.

Pattern 2—xylem of ventral vasculature and fruit tip slightly labeled, pod lateral walls unlabeled.

Pattern 3—xylem of ventral and some lateral strands labeled but dorsal strands and seeds unlabeled; some fruits accumulating dye subepidermally in lateral regions of their pod walls, other fruits lacking such labeling.

Pattern 4—dye in xylem of ventral, lateral, and outer dorsal strands; marked subepidermal accumulation in lateral pod wall; inner dorsal vasculature and seeds unlabeled.

Fruits were always heavily labeled at night (all of 25 observations) in winter, mostly exhibiting pattern 4. Pattern 4 was also common in early morning during winter (16 observations), though patterns 2 (nine observations) and 3 (eight observations) were also encountered. There was little evidence (only one of 23 observations) of positive intake through xylem (patterns 1 or 2) from late morning to late afternoon of sunny days in winter, but late morning or afternoons of cloudy days in winter sometimes (four of 21 observations) showed marked intake of dye (patterns 3 or 4).

Fruits of all ages failed (none of 63 observations) to take up dye (pattern 1) from 10 h to dusk during summer. Intense dye uptake (patterns 3 or 4) in summer was usually restricted to the late hours of the night (20 of 51 night time observations showing patterns 3 or 4).

Dye was never observed to enter the inner dorsal strands (Fig. 3, DL_1, DR_1) of a fruit or to penetrate seeds deriving their xylem supply from these strands. Where no dye had entered a fruit, the peduncle was labeled either throughout its length or only in its lower region.

Feeding of [^{3}H]Inulin and ^3H_2O to Cut Fruiting Shoots through the Transpiration Stream (Table II). Feeding of [^{3}H]inulin to winter-grown plants in the early morning labeled pod, but not seeds within 20 min (Table II). Shoots fed concurrently with acid fuchsin also showed ready acceptance of dye by fruits, again suggesting xylem (apoplastic) uptake. By 4 h, the ^{3}H of the fed

FIG. 4. Anatomical features of the vasculature of pod and seed stalk of fruits of cowpea (*V. unguiculata* cv Vita 3). A, Transverse section of dorsum of pod showing right (DR) and left (LR) sets of longitudinal xylem strands (xylem elements marked in black), bands of outer phloem OPV (outlined), protective fiber cap (FCD), and inner bands of phloem (IP) connecting across inside the xylem strands DR and DL. The sectioned seed stalk (SS) has a xylem (XSS) connection with the inner xylem strands of the dorsal vasculature DR, and phloem (PSS) connections with the inner (IP) and outer (OPV) phloem of the right hand half of the dorsum (× 75). B, Transverse section of part of side wall of pod showing lateral vein network (PWL) lying outside pod fiber layer (PWF) (× 50). C, Longitudinal section of seed stalk, showing continuous xylem (XSS) and phloem (PSS) supply to seed (× 270). D, High power transverse view of part of pod wall dorsal vasculature showing outer phloem (OP), dorsal xylem strand (X), internal phloem (IP), and part of xylem supply (XSS) to seed. Cells identified as sieve elements are marked with a central dot (× 310). E, Transverse section of venter of pod showing right (VR) and left (VL) sets of xylem strands (marked black), bands of outer phloem (OPD) (outlined), and fiber cap (FCV) protecting vascular tissue (× 75).

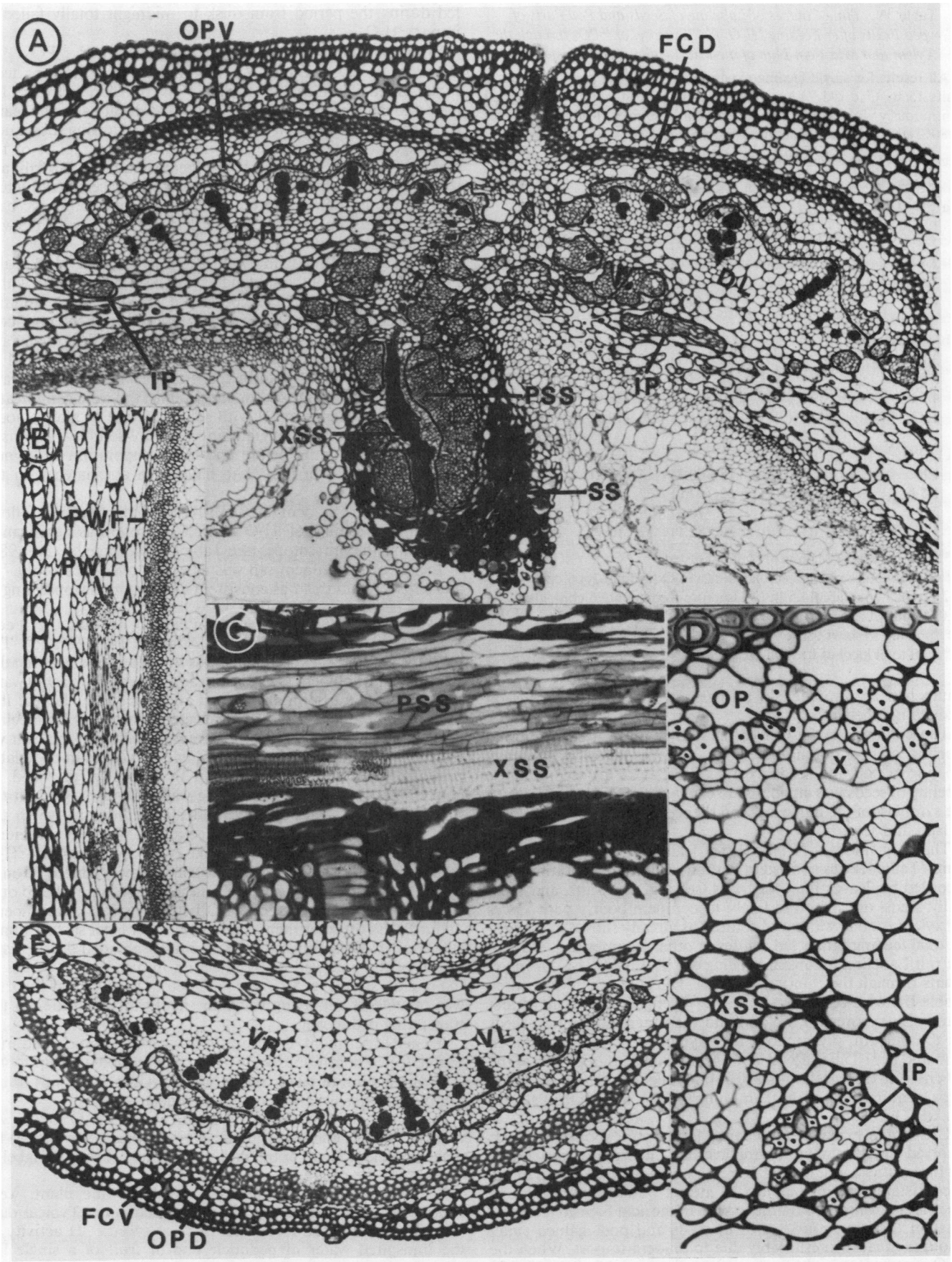
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Table IV. *Time Courses of Labeling of Seeds and Pod Parts of Cowpea Fruits after Feeding ^3H_2O, [^{14}C]Sucrose, or $^{32}PO_4$ through the Xylem to a Mid Vein Flap of the Mid Leaflet of the Nurse Leaf*

All results for single feeding experiments on single plant with study fruits 15 to 17 d old. Winter-grown plants, fed at noon and supplied continuously with isotope. See Table I for dose rates. Radioassays refer to 80% ethanol-soluble fraction of fruit tissues.

Label Fed/Times of Harvest	Pod Dorsal[a] Strip	Seeds	Pod Lateral Walls	Pod Ventral Strip
(a) ^3H_2O				
1.75 h	19[c] (4.5)[d]	23[c] (22.4)[d]	25[c] (68.7)[d]	33[c] (4.7)[d]
2 h[b]	23 (2.4)	8 (15.4)	30 (77.6)	50 (4.5)
2.5 h	59 (9.8)	13 (29.1)	13 (47.6)	99 (13.6)
3.5 h	76 (7.2)	49 (45.6)	19 (41.9)	41 (4.3)
4.5 h[b]	24 (7.7)	15 (49.0)	7 (38.2)	22 (5.0)
(b) [^{14}C]Sucrose				
2 h[b]	76 (3.5)	80 (63.1)	27 (30.1)	84 (3.3)
3.5 h	74 (7.9)	62 (64.6)	9 (21.5)	51 (6.0)
4.5 h	190 (5.0)	230 (63.7)	52 (23.9)	380 (7.4)
5 h[b]	150 (3.1)	270 (75.5)	98 (19.9)	270 (1.5)
(c) $^{32}PO_4$				
1 h	28 (6.9)	18 (60.8)	4 (17.2)	57 (15.1)
1.5 h	29 (8.3)	12 (67.9)	7 (19.2)	18 (4.6)
2 h	35 (5.2)	29 (70.8)	9 (20.6)	26 (3.4)
3 h	63 (5.0)	59 (82.1)	12 (10.9)	25 (2.0)

[a] See Figure 3 for regions of fruit harvested.

[b] Cryopuncture phloem sap labeling: ^3H_2O feeding, 1006 cpm/μl sap at 2 h; 1357 cpm/μl at 4.5 h; [^{14}C]sucrose feeding, 6696 cpm/μl at 2 h, 19,085 cpm/μl at 5 h.

[c] dpm/mg fresh wt tissue.

[d] % of total label of fruit, in parentheses.

inulin had accumulated in all parts of the pod; but seeds remained unlabeled.

The comparable ^3H_2O feeding study (Table II) also showed labeling of pod but not seeds at 20 min, followed by heavy labeling of pod and seeds by 4 h (Table II). This second phase labeling of seeds was attributed to exchange of labeled water with the symplast of the nurse leaf and thence by mass flow in phloem to fruit and seeds.

The very intense ^3H labeling of leaflets in comparison with fruits (Table II) was consistent with the higher transpiration rates recorded for leaves than for fruits (see Fig. 1). Fruits, and especially seeds, are therefore likely to compete poorly with leaves for xylem mobile water and solutes, as already shown in transpirational feeding of labeled solutes in other legumes (*e.g.* see 7).

Fruit Labeling following Feeding of $^{32}PO_4$ and ^3H_2O to Intact Plants through the Rooting Medium. The data for $^{32}PO_4$ feeding (Table III) were consistent with the ^{32}P entering the fruit at first mainly by xylem into the pod and then increasingly into seeds via the phloem, presumably after xylem to phloem transfer in stem and leaf. Thus, lateral walls of the pod acquired 90% of the ^{32}P entering during the first 30 min, while, over the next 1.5 h, seeds acquired significant amounts (40%) of the fruit's total intake of label.

The parallel experiments feeding ^3H_2O via the rooting medium involved night or daytime applications during summer. When fed in the afternoon, fruit walls remained unlabeled for up to 2.5 h, consistent with an inability to attract water directly through the xylem during the daytime as seen in the acid fuchsin feedings. Later in the time course (5–8 h) seeds and pods gained small amounts of label, presumably due to phloem import. When the ^3H_2O was fed at night certain fruits became labeled (18–103 dpm/g fresh weight) within 1 h of feeding (3.00 AM) while others fed during the period from dusk to midnight totally failed to import ^3H_2O.

Fruit Labeling after Feeding of Leaf Flaps with $^{32}PO_4$, [^{14}C] Sucrose, or ^3H_2O (Table IV). The mid vein flap feeding technique, already utilized in studies of N transfer to fruits (10), aimed to label initially the apoplast of the distal parts of a leaflet through the transpiration stream, and then, following subsequent exchange of labeled solute or water with the symplast of this region of the leaflet, to label the phloem stream serving fruit and seeds. Test applications via leaf flaps of the tracers acid fuchsin and [^{3}H]inulin demonstrated conclusively that apoplastic back flow from fed to proximal parts of the leaflet was negligible with this feeding technique.

Leaflet flap feeding with $^{32}PO_4$, [^{14}C]sucrose, and ^3H_2O (Table IV) led to all parts of the subtended fruit becoming labeled within 1 to 2 h. Throughout the courses of labeling, seeds attained higher specific radioactivities than lateral parts of the pod, consistent with a phloem-based distribution by mass flow (water and solutes) from the fed leaf. In such a system, seeds would be expected to comprise the principal sink for phloem-borne water and solutes. The ^3H_2O experiments (Table IVa) were complicated by possible exchange of ^3H with solutes before or after phloem loading. This proved negligible since ethanol-soluble fractions of tissues assayed for ^3H before and after evaporation to dryness showed over 95% of the ^3H to have been associated with the water of the extract.

Cryopuncture phloem sap (see 10) collected from fruits following leaf flap feedings of ^3H_2O and [^{14}C]sucrose, was significantly enriched with both isotopes (see Table IV, footnote b). Over 98% of the ^3H of the phloem sap was as water, over 90% of the ^{14}C as sucrose. Again this was consistent with mass flow operating to phloem between leaf and fruit.

Labeling of Transpired Water of Peduncle or Fruit after Feeding ^3H_2O via Leaf Flap, Root, or Attached Fruit (Fig. 5). The objective of these experiments was to determine the relative significance of phloem (symplastic) and xylem (apoplastic) water as sources for transpiration of fruit and peduncle. The leaf flap feeding technique (Fig. 5A) was selected as a means for labeling principally the symplastic compartment of water in the fruit via the phloem. Root feeding of ^3H_2O (Fig. 5B) was assumed to label primarily the apoplast of the fruit or peduncle, provided, of course, that the fruit was attracting water through xylem at the time of feeding. Alternatively, under conditions in which the fruit, but not its peduncle, was meeting its water requirements through symplastic (phloem) uptake, root application of ^3H_2O would possibly lead to the peduncle being heavily labeled through xylem, while the symplast of the fruit would become labeled only after secondary exchange of ^3H_2O between xylem and phloem. A third class of experiment, involving direct local surface application of ^3H_2O to fruits, through a mildly abraded dorsal distal region of the pod wall or by injection into the distal tissues of the pod dorsum (Fig. 5C), was designed to test whether the fruit was capable of supplying water in significant quantities to the transpiring peduncle.

In each experiment, the transpired water of peduncle and fruit was collected continuously (2–6 h harvest periods) for up to 4 d after application of label. Specific ^3H activities (dpm/ml) of the samples of transpired water were then determined.

In the flap and root feeding experiments, the ^3H_2O source was removed during the experiments (times indicated in Fig. 5) by detaching the fed leaf, or by thorough flushing of residual labeled water from the rooting medium.

Six replicate experiments, each involving one plant, were conducted using each type of labeling technique. Typical data (Fig. 5) are expressed as diurnal plots of specific ^3H activity of the transpired water of peduncle and/or fruit of a single fed plant.

Flap feeding of ^3H_2O (Fig. 5A) labeled the transpired fluid of

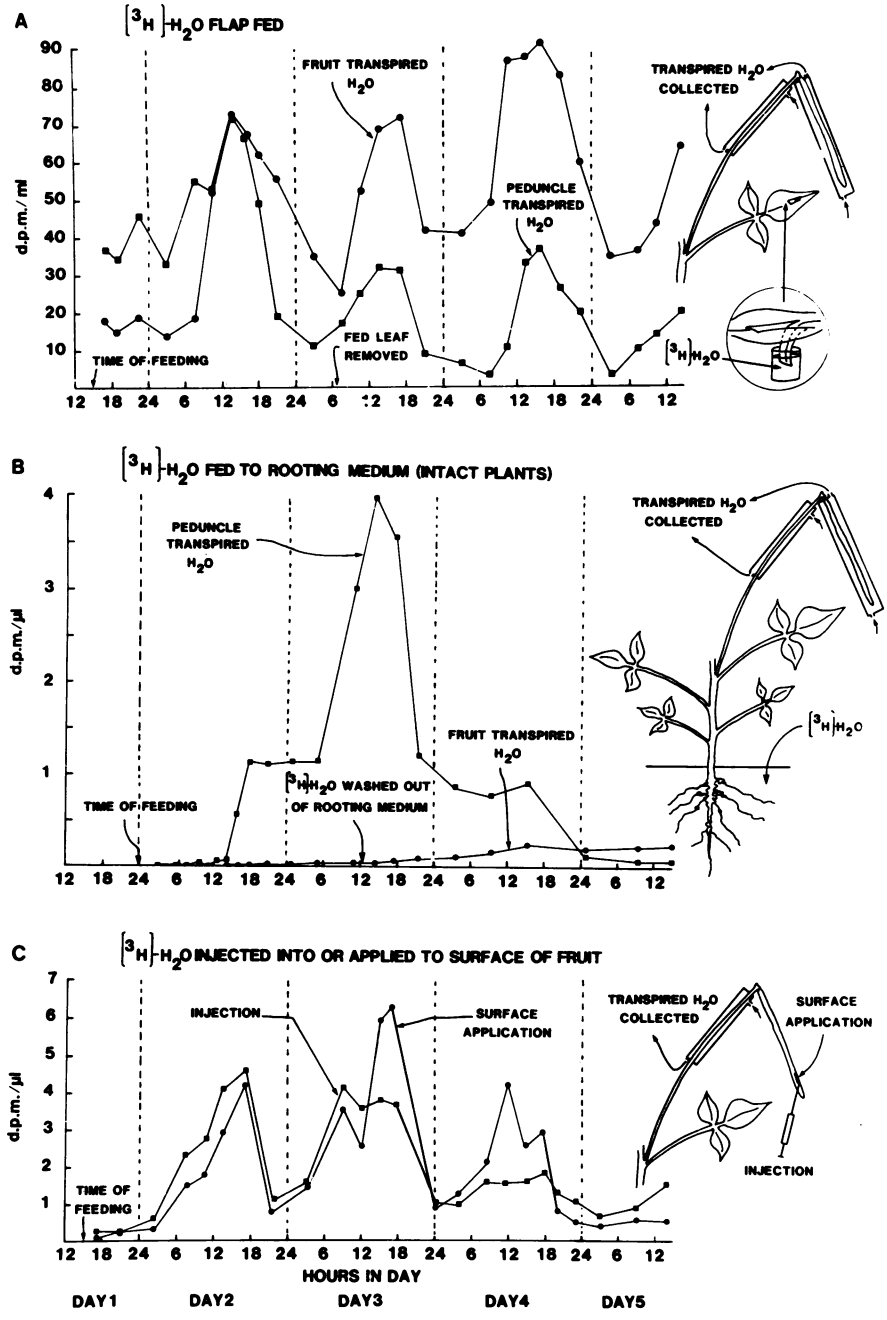

FIG. 5. Diurnal changes in specific ^3H activity of the transpired water of attached first-formed fruits and peduncles of fruiting intact plants of cowpea (*V. unguiculata*) after feeding of ^3H_2O to a mid-vein flap of the blossom leaf (A), to the rooting medium (B), or to the fruit by injection or surface application (C). The data relate to single fed plants. Times of feeding of ^3H_2O are indicated. The fed leaf was removed halfway through the experiment in A, and residual labeled water was washed out of the rooting medium at the time indicated in B. Note the marked diurnal rhythm in specific activity of transpired water of peduncle and fruit in A and C.

fruit and peduncle within 2 h. The specific ^3H activity of the transpired water of the peduncle was at first higher than that of the fruit, but, by 24 h, this had reversed and, towards the end of the experiment (96 h), fruit-transpired water was consistently of several times higher radioactivity than that of the peduncle. Coincident diurnal rhythms were evident in the specific ^3H activities of transpired water of peduncle and fruit for the last 3 d of the experiment (*i.e.* for 54 h after removal of the fed leaf). Peak specific radioactivities of transpired water during the day were assumed to denote times of greatest reliance on phloem-borne (symplast) water, minima in respective specific activities at night to coincide with periods when both peduncle and fruit were most active in intake of root-derived (unlabeled) water through the xylem.

The high specific radioactivity of the transpired fluid of fruits was consistent with their having acquired a large intensely labeled pool of phloem-borne water early in the time course of the experiment. This water clearly continued to act as a major source for fruit transpiration for several days, after removal of the fed leaf, and may even have passed back out of the fruit in the xylem to contribute to the transpiration of the peduncle. Assay of fruits for ^3H at the termination of the experiment showed the specific ^3H activity of their tissue water to be within ±10% of that of their currently transpired water, indicating that during daytime a fruit was transpiring almost exclusively its own reserves of water.

Root feeding of ^3H_2O (Fig. 5B) differed from leaf flap feeding in labeling the transpired water of peduncles to much higher specific activity than that of fruits. Peduncle transpired water showed a marked reduction in labeling following removal of ^3H_2O from the rooting medium, suggesting peduncles contained only a small reserve of water relative to their daily needs. The low, but continually increasing specific radioactivity of the transpired water of fruits indicated little direct uptake by the fruit

through the xylem, as expected under hot summer conditions. Any labeling which did occur presumably resulted from slow, secondary exchange of ^3H_2O with the phloem. Surface applications of ^3H_2O to top mid surface of fruits or injection of ^3H_2O into their distal dorsal tissues (Fig. 5C) led to significant labeling of the transpired water of peduncles within 3 h. Peak ^3H specific activities were attained at 27 h (surface application) or 51 h (injection). Diurnal fluctuations in specific ^3H activity of peduncle transpired water were evident, higher radioactivities in the day (Fig. 5C) suggesting proportionately greater back flow of water from fruit to peduncle than at night.

Note that in this and earlier sections of the paper all ^3H_2O labeling patterns and time courses indicate strongly that only very slow and ineffective exchanges of water occur between symplastic and apoplastic compartments of an organ, tissue, or transport pathway. The interpretations presented here are based on such an assumption, and clearly run contrary to the widely held view that recently acquired water equilibrates rapidly with existing pools of water in all compartments of a plant tissue, irrespective of their locations.

Further evidence of back flow of water from fruit to tissues of the parent plant was obtained by assaying fruit and peduncle tissues for labeled water at different times after application of ^3H_2O at noon to the distal surface of fruits. Sequential harvests of the labeled fruits showed that by 1 h, 0.1% of the applied ^3H_2O had reached the peduncle; by 2 h, 3.4%; and by 4 h, 9.4%. Feeding of acid fuchsin to the cut distal tip of fruits provided similar evidence of back flow, the xylem of the top half of the peduncle being labeled within 90 min and dye having spread by 4 h to the vascular and subepidermal regions of the whole peduncle. In certain plants, dye eventually reached the subtending leaf.

CONCLUSIONS

The anatomical and tracer studies on cowpea fruits presented here support the concept of diurnally reversing xylem flow of water between fruit and parent plant. The following features are envisaged for the system:

(a) Xylem intake by pods occurs principally at night when transpiration loss of water from the wall exceeds current intake of water by mass flow in phloem.

(b) Conversion by seeds of phloem stream solutes into insoluble reserves generates a continuous excess of water, day and night. This exits via the xylem, whence it may return directly to the plant via the inner dorsal pod strands or move laterally to sites of transpiration in the pod wall.

(c) Back flow of water in xylem from whole fruit to plant occurs mainly late in the day, when phloem intake of water is exceeding transpiration loss of the pod.

(d) Fruit and peduncle utilize pools of symplastic (phloem-derived) water and recently arrived apoplastic (xylem) water for their transpiration. Fruits are generally much more dependent on phloem-derived water than are peduncles.

(e) Fruit and peduncle rely more heavily on symplastic reserves of water for transpiration in the day than at night.

(f) Phloem-derived water exchanges slowly with the whole symplast of the fruit and is subsequently used in fruit transpiration over a period of several days after arriving in the fruit.

(g) Water flowing out of the fruit in the xylem is used for peduncle transpiration, especially in the day.

The hypothesis of diurnally reversing xylem flow depends on there being a much greater diurnal amplitude in translocatory intake of water than in transpiration loss. This aspect remains to be tested experimentally.

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