

SUPPLEMENTAL DATA

Supplemental Table 1. Neither DMSO nor sucrose contributed to apparent flavonoid movement rates.

Condition ^a	DMSO/No Suc	EtOH/No Suc	EtOH/Suc	H ₂ O/No Suc
Compound	mm ^b			
N	12.6 ± 0.9 ^d	16.2 ± 1.2 ^e	16.7 ± 2.2 ^e	11.1 ± 0.6
DHK	12.5 ± 1.6 ^c	n.d. ^f	n.d.	n.d.
DHQ	12.3 ± 0.7 ^d	n.d.	n.d.	n.d.
K	9.6 ± 0.5 ^c	9.6 ± 0.3	8.7 ± 0.4	n.d.
Q	10.9 ± 0.7 ^d	9.7 ± 0.4	10.5 ± 1.0	n.d.

^a The solvent the indicated flavonoids were dissolved into and the presence or absence of sucrose in the agar cylinder were varied. The first compound is the solvent and the second compound is the amount of sucrose in the agar. DMSO = dimethyl sulfoxide; EtOH = ethanol.

^b Average and SE from 2 combined independent experiments ($n \geq 10$). Compounds were applied in the agar cylinder at the root tip for 1 h, DPBA stained, and the fluorescence distance was measured from the application site.

^c Statistical analyses compare *tt4-1* seedlings incubated with or without sucrose in the agar stab using 2-tailed Student's *t*-Tests with equal or unequal variance depending on F-test for variance: $0.05 < P > 0.001$; ^d $P < 0.001$

^e Statistical analyses compare *tt4-1* seedlings DMSO/no sucrose to EtOH/no sucrose. $P < 0.05$ using 2-tailed Student's *t*-Tests with equal or unequal variance depending on F-test for variance.

^f n.d. not determined

Supplemental Table 2. Comparison of the absorption maxima of the exogenously applied aglycones according to HPLC.

Compound	Absorption maxima (nm)
N	sh ^a 220; 230; 290; sh 315
DHK	sh 220; 230; 288; sh 315
DHQ	sh 220; 230; 288; sh 315
K	sh 220; 230; 265; sh 335; 365
Q	sh 220; 230; 255; sh 340; 370

^a sh = shoulder

Supplemental Table 3. Flavonoid fluorescence localisation differs with flavonoid compound and application site.

Compound	Application Site	Localisation Site
N	RT ^a	Epidermis
	MR ^b	Pericycle
DHK	RT	Cortex
	MR	Pericycle
DHQ	RT	Entire root
	MR	Pericycle and cortex
K	RT	Cortex
	MR	Not taken up
Q	RT	Epidermis
	MR	Not taken up

CLSM optical sections were generated and optically cross-sectioned to determine flavonoid fluorescence localisation following DPBA staining.






^aRT = root tip

^bMR = mid-root

Supplemental Figure and Movie Legends

Supplemental Figure 1. Kaempferol and quercetin generate intense fluorescence in the presence of DPBA. Spectrofluorometric analysis and integration for the area under the respective curves resulted in the following areas: N- 359.8; DHK- 2,382.2; DHQ- 1,425.6; K- 87,961.3, and Q- 155,969.0. Thus, Q fluorescence is 2 times that of K and 433 times N. Integration was performed using Origin 7.0 calculus integration option using zero as the baseline. Abbreviations: naringenin (N), dihydrokaempferol (DHK), dihydroquercetin (DHQ), kaempferol (K), and quercetin (Q). The compounds were conjugated with DPBA and excited at 488 nm through a 5 nm slit. Emission data were collected through a 5 nm slit.

Supplemental Figure 2. Supplying aglycones at *tt4-1* root tips resulted in downstream flavonoid biosynthesis and basipetal movement. The indicated flavonoid compounds (100 μ M) were applied at the root tip for 5 or 60 min, DPBA stained, and photographed. The controls (A and G) show only dim background fluorescence from sinapate esters. A-F are following 5 min incubations and G-L are after 1 h incubations. The arrow in E indicates DPBA fluorescence consistent with K formation at the root tip. Abbreviations: DHK: dihydrokaempferol; DHQ: dihydroquercetin; K: kaempferol; N: naringenin; Q: quercetin. The scale bar = 100 μ m.

Supplemental Figure 3. The aglycone standards have unique absorption spectra. These spectra along with the indicated elution times were used to confirm the unknown peak identities. The traces are: brown  – DHK; green  – DHQ; blue  – Q; black  – N; and red  – K.

Video S1-S3, and S5. Adding N, DHK, DHQ, or Q near the root tip of a *tt4* seedling resulted in the nearly immediate formation of quercetin fluorescence in specific cells in the distal elongation zone. The images were collected every 15 s for 10 min after adding the aglycones. The times are in the lower left hand corner.

Video S4. Adding K reveals a possible reverse reaction through FLS. The times after adding naringenin are supplied in the lower left hand corner. As this reaction was slower, the images were taken every 1 min over a total of 25 min.