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Sectoriality in xylem connections between the bunch and leaves of the grapevine (*Vitis vinifera*) shoot

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Abstract

It is not clear how the xylem of plant organs with low transpiration rates, such as the grapevine (*Vitis vinifera* L.) bunch, are anatomically linked to surrounding leaves. The aim was to test the hypothesis that xylem connections to the grapevine bunch are sectorial using an apoplastic dye and a radioactive membrane-permeable nutrient analogue. The transport of eosin and pertechnetate ($^{99m}TcO_4^-$) revealed that bunches bearing unripe berries have xylem connections with primary leaves located on the same side of the shoot (common orthostichy) as the bunch. These connections also exist with secondary shoots arising from axillary buds in the same orthostichy as the bunch. These connections can include leaves in both orthostichies of the secondary shoot. Connections between the bunch and primary leaves in the opposite orthostichy are relatively uncommon. The challenge for future research is to determine the degree to which these connections are functional under normal physiological conditions.

1. Introduction

In the present research it is hypothesised that there is a sectorial supply of xylem sap to the bunch stem of the grapevine (*Vitis vinifera* L.) defined by the vascular architecture of the primary shoot. A connection from the bunch to the leaves of the shoot seems likely because previous studies demonstrate that the pre-veraison grape berry is a source of water for the transpiring grapevine canopy (Greenspan et al., 1994) and apoplastic dyes are transported from berries into leaves (Keller et al., 2006) and nearby internodes (Tilbrook and Tyerman, 2009). Due to the sectorial phloem transport of assimilates to the bunches of *V. vinifera* and its hybrids (Hale and Weaver, 1962; Motomura, 1990) it is expected the bunch also has xylem connections to specific leaves on the shoot. This would be in agreement with observations of

sectorial apoplstic dye transport in the axial stele of the *V. vinifera* primary shoot (Theiler, 1973; Stevenson et al., 2004). Therefore, the objective of this research is to identify whether there are sectorial xylem connections to the pre-veraison bunch stem and to assess the relationship between these connections and the orthostichies of the *V. vinifera* shoot. *V. vinifera* is an excellent subject for this investigation because xylem transport is of physiological significance to the development of its fruit (Rogiers et al., 2000; Rogiers et al., 2006).

2. Materials and methods

2.1. Labelling bunches with dye

The xylem-mobile dye, eosin, (eosin yellowish, Chem-Supply, Gillman, SA, Australia) was introduced to the bunch stem of rooted one-bud cuttings of 'Chardonnay' grapevines (clone I10V1) using a cotton wick. The plants (six plants, one bunch per plant) were in their second season of vegetative growth, pruned to two shoots and maintained in 2 L pots in a naturally-lit glasshouse (30/25 °C, 40/50 % relative humidity day/night). In the glasshouse, shortly after dawn (6 AM), a cotton thread moistened with deionised water was pushed through the distal end of the peduncle (Pratt, 1971) of a pre-veraison bunch (35 – 40 days after flowering, DAF) with a needle. One end of the thread was allowed to hang freely to promote evaporation and the other end was secured in a micro-centrifuge tube containing aqueous eosin (0.1 % w/v, osmotic potential of -0.003 MPa determined with an Advanced Instruments model 3320 osmometer, Norwood, Massachusetts) and wrapped in aluminium foil. The shoot carrying the labelled bunch was sampled approximately 27 h later. Hand-sections were taken of each internode and of secondary shoots or petioles occurring at each node. The sections were placed on a

glass slide, examined using a stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland, model MZ16) and a digital microphotograph was obtained using transmitted and green light (Leica DSR filter, excitation 545 ± 15 nm, barrier 620 ± 30 nm). These images were examined for the presence of eosin after digitally merging them using image analysis software (ImageJ, Rasband 1997-2012, Schmidt 2002). Only strong fluorescence (> 25 greyscale units in the unprocessed green light image) was attributed to the dye because trials indicated unlabelled plant tissues exhibited weak autofluorescence under green light.

2.2. Matching the osmotic potential of berry juice and dye with PEG

The wick experiment described above was repeated with another eight potted grapevines (cv. Shiraz, clone PT23/N/GRIF). Two days prior to labelling, three berries were sampled from each bunch to be labelled. The berries were pooled and crushed to extract juice which was analysed for its osmotic potential. This sampling and analysis was done in triplicate. The osmotic potential of the eosin solution was then adjusted to the mean osmotic potential of the extracted juice using polyethylene glycol 4000 (Ajax Finechem, Sydney, NSW, Australia). The dye was then administered and observed as described above.

2.3. Labelling bunches with pertechnetate

The above-mentioned wick method (Supplementary Fig. 1) was used to label the pre-veraison bunches (35 – 45 DAF) of potted grapevines (cv. ‘Shiraz’) with pertechnetate ($^{99m}TcO_4^-$, 1 GBq, ca. 0.2 % saline solution after dilution in deionised water, osmotic potential of -0.2 MPa). The radionuclide is xylem-mobile (Clarke et al., 2012), possesses a half-life (6 h) and in the form of pertechnetate exhibits

transport characteristics of a micronutrient, possibly molybdate (Bennett and Willey, 2003). The plants (four, one bunch per plant on a shoot with at least eight nodes) were in their fourth season of vegetative growth, pruned to four shoots and maintained in 40 L pots in a naturally-lit glasshouse (24/23 °C, 55/50 % relative humidity day/night). The plants were transferred to the laboratory (21/16 °C day/night, 55 % relative humidity) the day prior to imaging. Plants used in this experiment were illuminated continuously using 100 W halogen lamps for approximately 28 h. The labelling commenced approximately 3 h after illumination had begun. The ^{99m}Tc distribution in the plant was detected scintigraphically (20 min acquisition, 256 × 256 pixel matrix) at the experiment end using a gamma camera (Philips Prism 1000, Picker, Cleveland) after removing the wick. Scintigraphic images (displaying total counts during the acquisition) were processed by first digitally truncating to approximately 500 counts. A colour palette was then applied to the image, which was then exported and digitally merged (Merge 2.0 freeware, www.graphicutils.com) with a digital photograph taken of the plant during scintigraphic acquisition.

2.4. Statistical analysis

An analysis of variance (GenStat release 15.0, VSN, Hertfordshire, UK) was used to assess the separate effects of orthostichy, organ type and direction (acropetal/basipetal) on the presence of dye or ^{99m}Tc. LSDs were calculated at 5 % significance. Means are presented ± one standard error.

3. Results

The primary leaves and secondary shoots most commonly stained with eosin were in the same orthostichy as the labelled bunch (Table 1, Figs 1 and 2). There was one

exception to this trend - dye was detected in the petiole of the primary leaf located at the same node as the bunch (Fig. 1). No significant difference was observed in the distribution of dye between primary leaves and secondary shoots or between organs located acropetal and basipetal of the labelled bunch (Table 1). In internodes, dye was always confined to vascular bundles within or immediately adjacent to the sector of vascular tissue characterised by the absence of the large vessels observed in other sectors/quadrants. When eosin was applied in a solution with the same osmotic potential as berry juice (-0.88 MPa) dye was confined to the labelled rachis (n = 5) or typically as far as the internodes immediately distal and proximal to the node of the labelled bunch (n = 2). On one occasion eosin was transported two internodes basipetal one internode acropetal to the node of the labelled bunch.

After labelling the peduncle of a bunch with $^{99m}TcO_4^-$, scintigraphy produced images showing the isotope was most often transported to primary leaves and secondary shoots arising from axillary buds in the same orthostichy as the labelled bunch (Table 1, Fig. 3). In one instance, xylem-mobile radiotracer was transported from a labelled bunch stem to three leaves occurring on sequential nodes within a secondary shoot, not just those occurring at every second node as often seen in the primary shoot. The identification of leaves to which ^{99m}Tc was transported from labelled bunches was aided by characteristic tracer accumulation patterns along the transport pathway (Fig. 4). A ubiquitous trend was the presence of ^{99m}Tc in the internodes that separate the bunch from the sink organ. The transport pathway was further delineated by relatively high concentrations of ^{99m}Tc in the first few centimetres basipetal to nodes but also immediately acropetal to the node and at the pulvinate (sensu Ellis et al., 2009) regions at the distal and proximal ends of petioles.

4. Discussion and conclusions

The transport patterns of membrane-impermeable dye and the gamma ray-emitting membrane-permeable nutrient analogue demonstrate there are extensive xylem connections between the *V. vinifera* bunch and primary and secondary leaves within the same orthostichy as the bunch. This is in agreement with dye injection experiments (Theiler, 1973; Stevenson et al., 2004). The dye observations suggest that these connections are closely associated with the ‘lateral’ sectors (sensu Stevenson et al., 2004) of the primary shoot. The present research provides the first evidence that, where a xylem connection exists between a grapevine bunch and secondary shoot, the connection can involve leaves in both orthostichies of the secondary shoot. Connections between the bunch and the opposing orthostichy of the primary shoot

were identified as relatively uncommon. Xylem flows are therefore strongly sectorial in the grapevine primary shoot, a constraint equally apparent in previous photoassimilate tracing studies (Hale and Weaver, 1962; Koblet, 1969) and predicted by the vascular anatomy of the grapevine shoot (Fournioux and Bessis, 1979; Stevenson et al., 2004). The observation of nutrient analogue accumulation in nodes (see also Hewitt and Gardner, 1956) and petiole ends in particular, provides the impetus for exploring refined techniques of tissue sampling for routine nutrient analyses.

The results obtained following the insertion of a wick through the bunch stem do not provide evidence that backflow from the bunch to other organs on the grapevine occurs in untreated plants. This is because the insertion and presence of the wick is expected to enhance the hydrostatic gradient between the site of tracer introduction (hydrostatic pressure close to atmospheric pressure) and organs elsewhere on the grapevine shoot that are generating tension in the xylem: backflow will therefore be artificially enhanced. This gradient is also enhanced by the weak osmotic potential of the dye and radiotracer solutions relative to the osmotic potential of pre-veraison berries. It is therefore notable that when the osmotic potential of the eosin solution was strengthened, the dye was not transported in detectable quantities typically more than an internode from the node of the labelled bunch. These considerations imply that the wick method may over-estimate the strength of hydraulic connections between the xylem vessels of the grape bunch and other organs on the grapevine shoot under normal physiological conditions. The degree to which the vascular connections identified here are functional in a vineyard setting will depend on spatial and temporal variations in the flow of xylem sap within a grapevine canopy, driven by tissue

hydration and osmotic status, transpiration and the hydraulic conductivity of the pathway.

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Figures

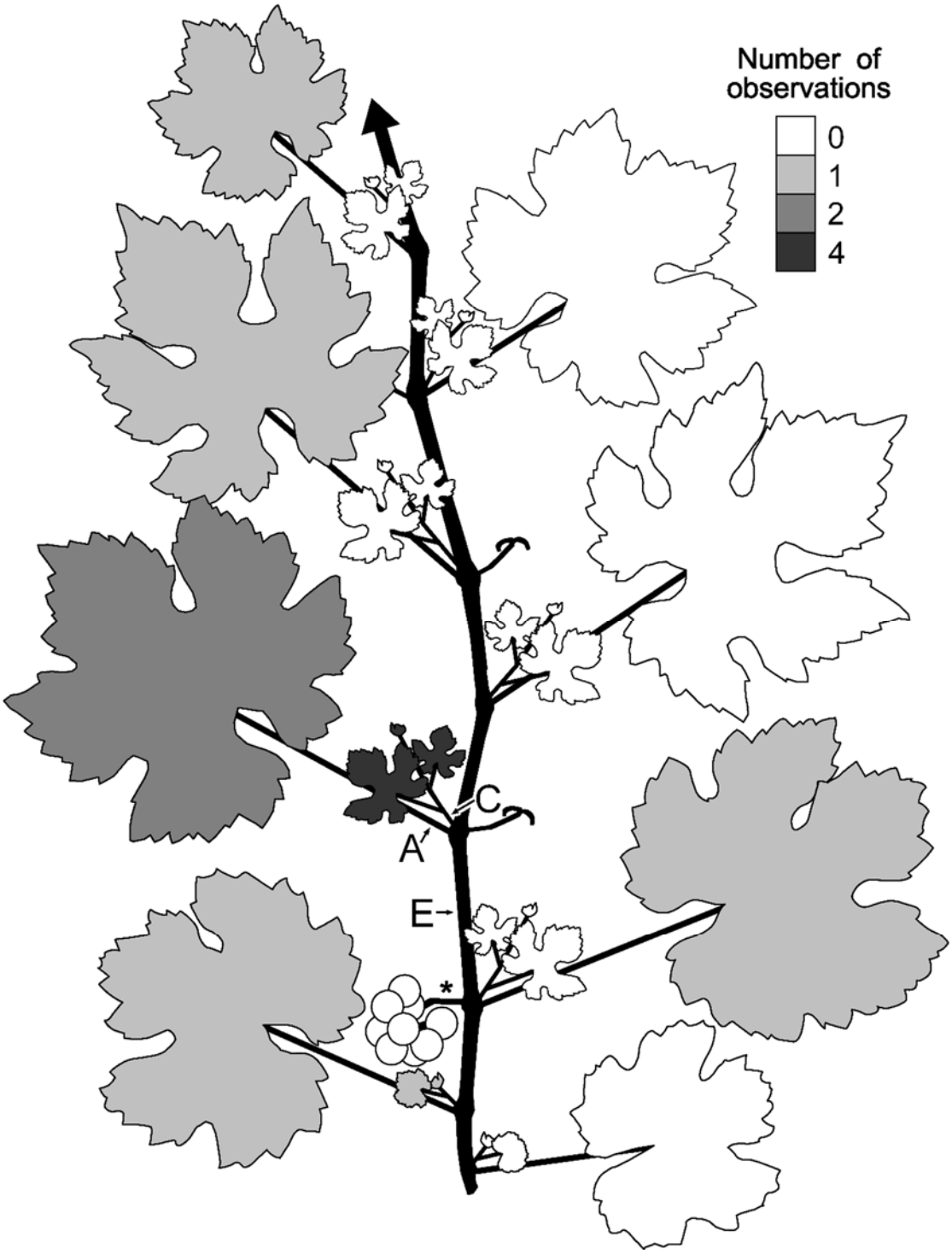


Figure 1. Sketch indicating eosin translocation from a cotton wick threaded through the peduncle of pre-veraison bunches on rooted ‘Chardonnay’ cuttings (*n* = 6). Results are shown for the basal eight nodes only. Approximate site of the wick is indicated by the asterisk (*). Letters correspond to the sample locations of A, C and E in Supplementary Figure 1. Shading of primary and secondary leaves (large leaf and

small leaves at each node, respectively) is based on cumulative data across all plants and is grey-scale coded to indicate the number of times eosin was observed in their petiole or shoot base, respectively. For simplicity, all leaves in the secondary shoot are grey-scale coded although the presence of dye was only confirmed in the shoot base.

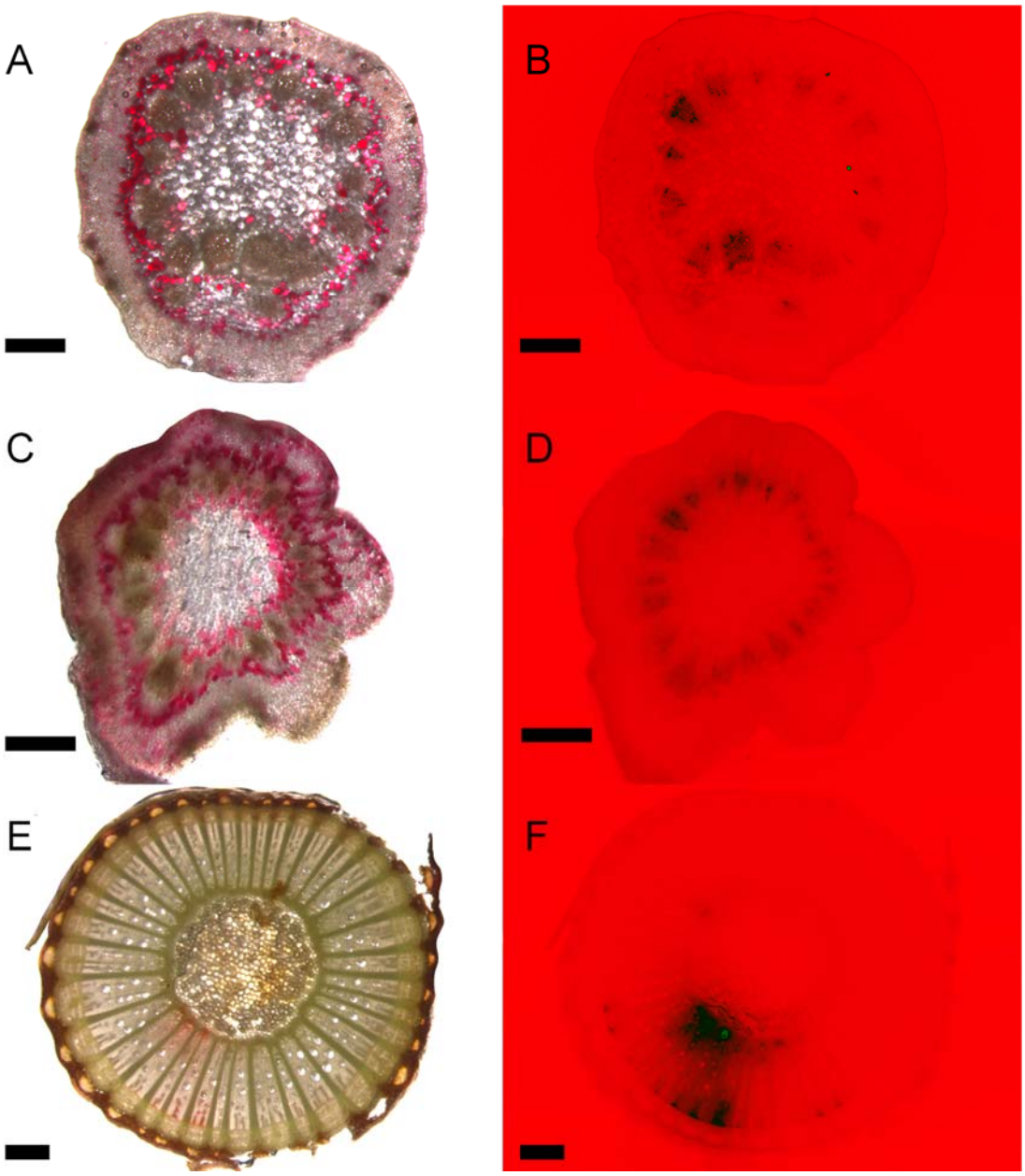


Figure 2. Examples of eosin staining in grapevine tissues (A, B) at the proximal end of a primary leaf petiole, (C, D) at the proximal end of a secondary shoot and (E, F) of a primary shoot internode. Images on the left were obtained under bright field illumination. The fluorescence images indicate the red pigmentation observed in the parenchyma cells bordering the vascular bundles in A and C is not associated with the presence of dye. Images on the right were obtained under green light and digitally manipulated (inverted in the red channel) to display areas of eosin staining as black.

All tissue sections were obtained from a single plant. These petiole, secondary shoot and internode samples were located on the plant immediately acropetal to the labelled bunch (as indicated in Figure 1). The scale bar is 0.5 mm wide.

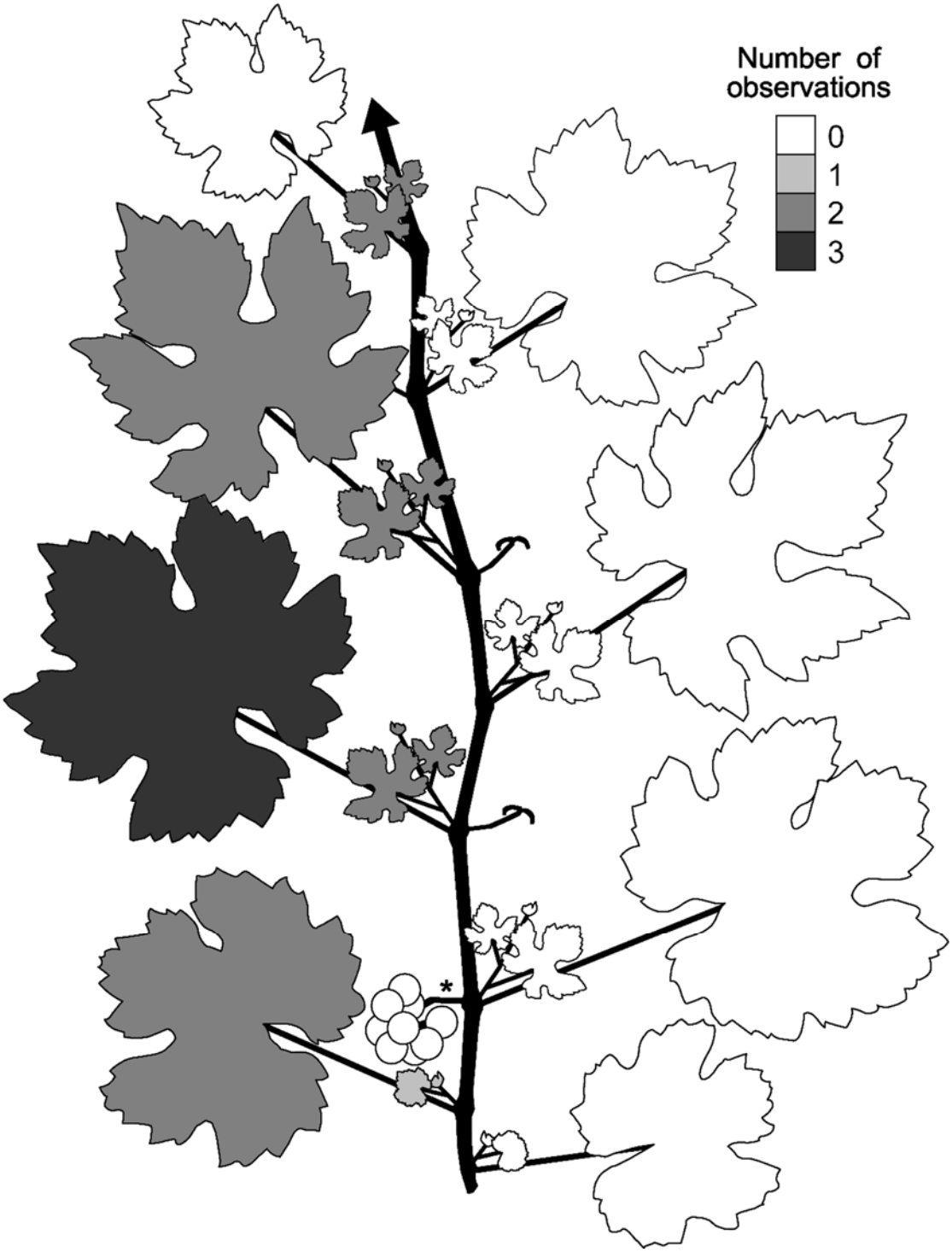


Figure 3. Sketch indicating ^{99m}Tc translocation from a cotton wick threaded through the peduncle of pre-veraison bunches on potted ‘Shiraz’ plants (n = 4). Approximate site of the wick is indicated by the asterisk (*). Results are shown for the basal eight nodes only. Grey-scale shading of primary and secondary leaves (large leaf and small

leaves at each node, respectively) is based on cumulative data across all plants. For simplicity, all leaves in the secondary shoot are grey-scale coded although ^{99m}Tc may not have been detected in multiple leaves.

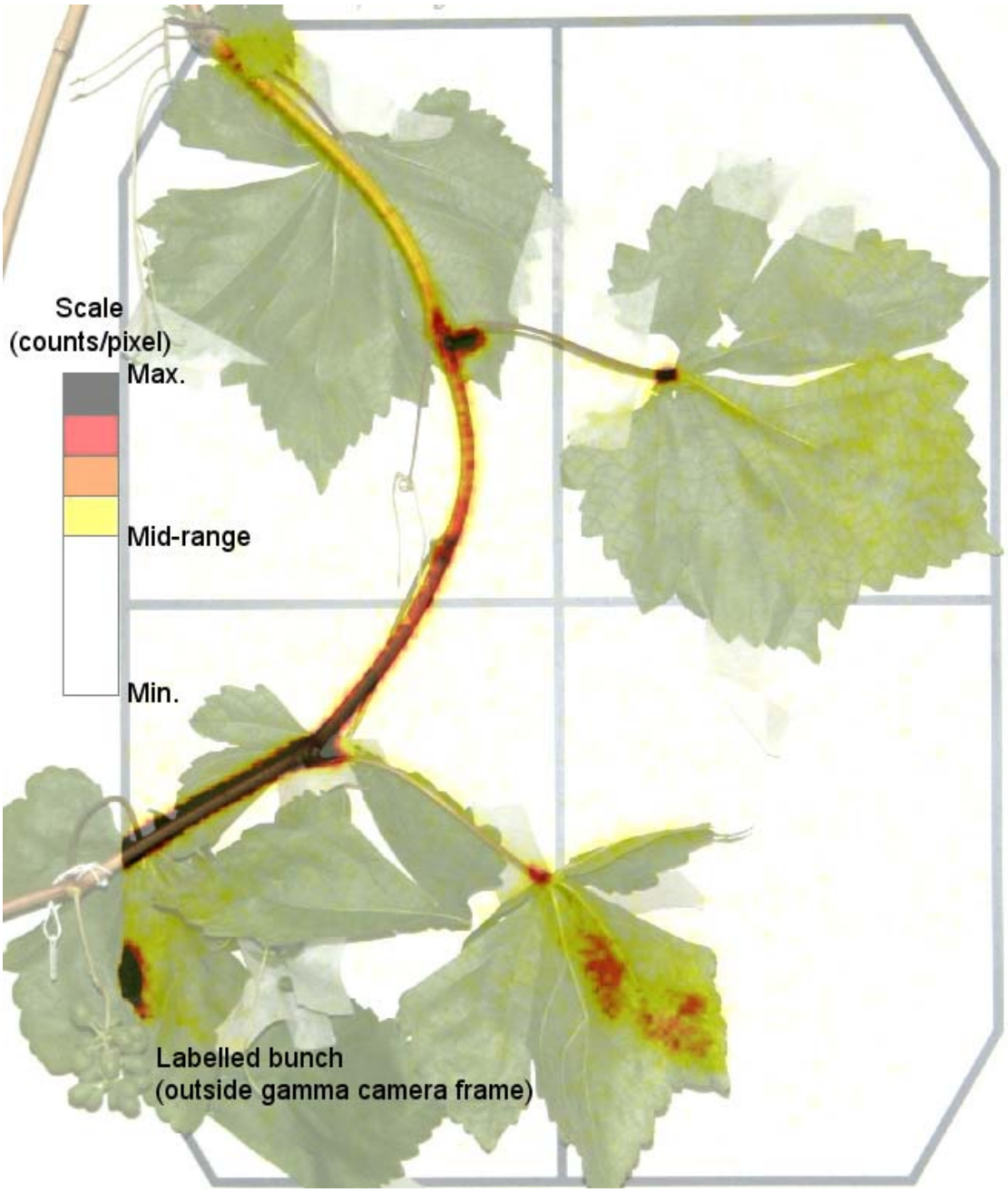
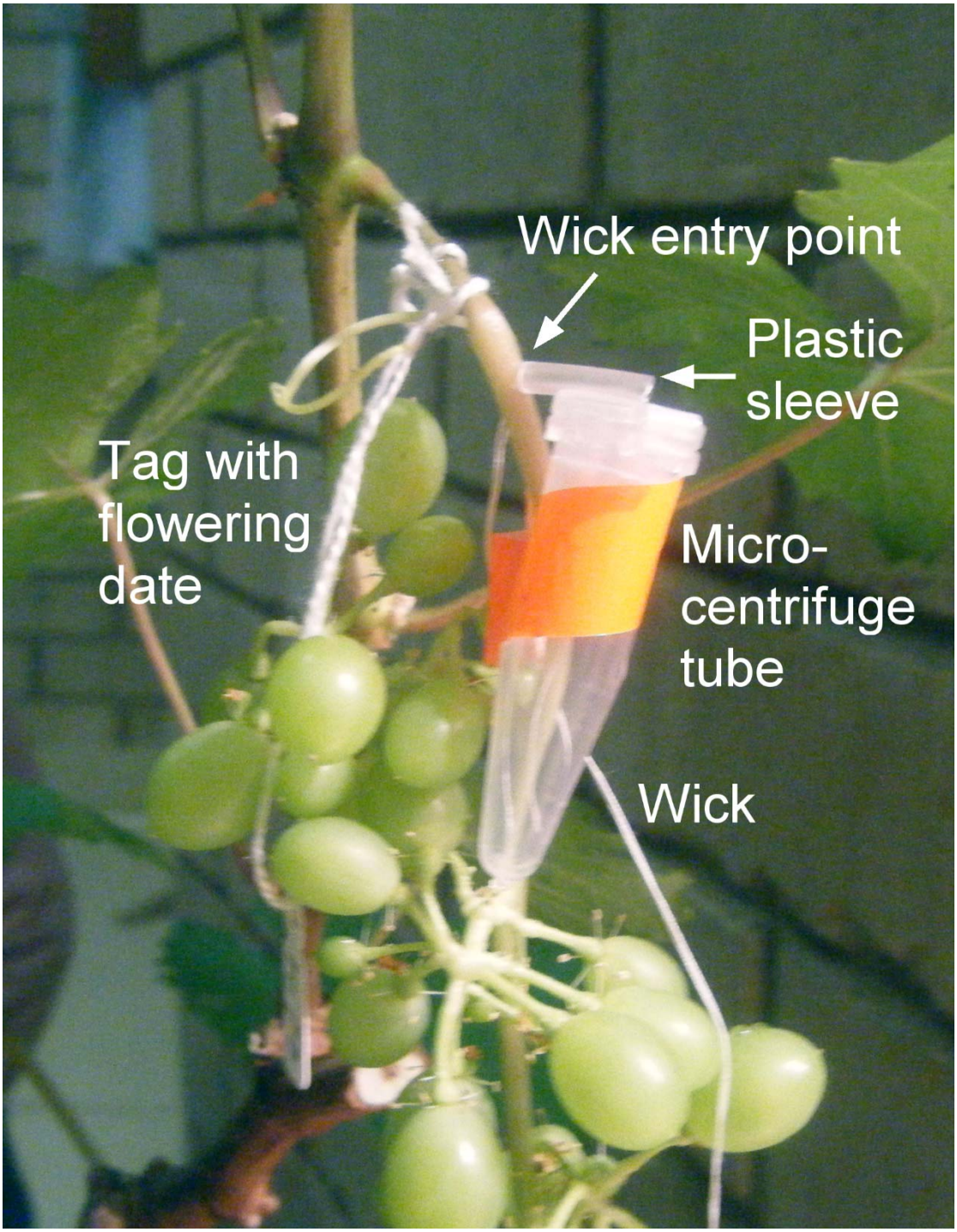


Figure 4. Photographic image, merged with a scintigraphic image, of a grapevine shoot labelled with $^{99m}TcO_4^-$ introduced via a cotton wick through the peduncle of the pre-veraison bunch. The scale was inserted into the scintigraphic image prior to merging and indicates how the counts per pixel (range of ca. 0 – 500) were colour-coded to depict the spatial distribution of gamma radiation. The labelled bunch can be seen in the bottom left of the image, outside the frame of the gamma camera. Counts

radiating out from the labelled bunch are an artefact resulting from relatively high radioactivity in the vicinity of the wick (removed prior to scintigraphic imaging).



Supplementary Figure 1. Digital photograph of a pre-veraison bunch being prepared for labelling with $^{99m}TcO_4^-$ via a cotton wick inserted in the peduncle. Upon exiting a hole pierced in the top of the microcentrifuge tube the wick is threaded through a plastic sleeve to minimise evaporation between the reservoir and tissue. The orange tape is used to secure the tube to the peduncle. At the time of the photo the radiolabel

was not yet placed in the microcentrifuge tube and the wick was not yet trimmed to avoid contaminating nearby surfaces, such as the berries.