Executive summary of Honours thesis project by Wesley Beelders

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Thesis title: Exploring the structure/function relationship of auxin analogues in plants

Previous work has shown that during normal berry development the endogenous auxin, 2-(1H-indol-3-yl) acetic acid (IAA), may inhibit berry ripening. IAA levels are high at flowering and decrease to low levels before the commencement of ripening (veraison). Treatment of berries with the synthetic auxin 2-(naphthalen-1-yl)acetic acid (NAA) can delay ripening more than IAA and the synthetic auxin-like compound 2-(benzothiazol-2-yloxy) acetic acid (BTOA) can delay ripening even further. Treatments with auxins appear to have little effect on the metabolite levels and flavour of wines made from the delayed ripening fruit. These properties make auxins potentially useful tools in controlling the timing of grape berry ripening and therefore the timing of fruit harvest. This would be valuable to the industry in managing fruit intake at the winery and possibly sugar/flavour profiles and berry ripening synchronicity. The ability to better manage fruit harvest and winery intake could reduce operating costs, reduce wastage of fruit and improve fruit 'quality' through enabling harvest to be determined by ripeness rather than by winery capacity concerns.

The aim of this project was to better understand why different auxins have different effects on ripening and to determine the aspects of the structure that are important for their effect. To achieve these aims seven new compounds which were variations on the structures of IAA, NAA and BTOA were synthesised. The synthesised analogues were used in two systems to ascertain how these structural differences affected biological function. The molecules targeted for synthesis are designed to test the importance of the ring structure and the side chain. An *in vitro* assay was used to determine if the structural changes affected the ability of grapevine IAA-amido synthetase enzymes (GH3 enzymes) to conjugate the synthesised auxin analogues to amino acids. The conjugation of auxins to amino acids by these enzymes inactivates their hormonal activity and is thought to control the level of auxins in berries. Those compounds that are not well conjugated may be more active in the plant due to possessing longer biological half-lives. The second test was designed to determine *in planta* auxin activity using root growth assays, this is a classical test for auxin activity. Apart from conjugation auxin activity in plants can be influenced by their ability to be transported within plant tissues by specific protein transporters and their ability to be recognised as auxins by receptor proteins that specifically bind the auxin and initiate the auxin signalling cascade. The changes in structure of the analogues may lead to a change in binding to the auxin receptors. An improved understanding of the structure/function relationships could allow the tailoring of auxin structures to provide new plant growth regulators that can delay grape ripening to different extents or provide auxins that affect ripening in slightly different ways.

Seven compounds based on the structure of auxins BTOA, NAA and IAA were synthesised. BTOA is not a substrate of the GH3 enzymes and the structural changes made to it caused no change in its ability to be conjugated. GH3-1 catalyses the conjugation of NAA with aspartic acid. However, the introduction of a sulphur or oxygen in the side chain to form NAA#1 or NAA#2, resulted in a lack of conjugation of these compounds. IAA is a substrate of GH3-1 and GH3-6 enzymes. Insertion of a sulphur atom into the side chain (IAA#1) caused no disruption in the ability of the compound to be conjugated. Thus in some cases it is possible to reduce the ability of auxins to be conjugated by simple changes to their structure. This may make them more stable in the plant and therefore more potent.

The compounds synthesised still have to have auxin activity in plants. *In planta* auxin activity of the various compounds was determined by measuring their effect on shortening of the main root of Arabidopsis plants grown on agar containing the putative auxins. The two BTOA analogues acted as auxins, however, the extent of main root growth reduction was less than that observed for BTOA. The changes made to NAA and IAA disrupted their ability to act as auxins. Interestingly, secondary effects such as root hair distribution were observed for all the compounds that acted as auxins. The difference in root hair distribution caused by BTOA#1 compared to BTOA#2 is of particular biological interest.

Improved grape ripening inhibitors need act as auxins to delay ripening, but not be conjugated by GH3 enzymes. Hence, the new BTOA analogues, BTOA#1 and BTOA#2 are potential candidates as grape-ripening inhibitors. Their subtly different effects in the root growth assay indicate that they may be useful in producing slightly different effects on grape berry ripening.

This work will be carried further by the team at CSIRO. The effects of these compounds in both the *in vitro* and *in planta* tests will be further defined and the effect of these compounds on berry ripening will be tested in the field. They will be useful scientific tools but may also have application in controlling berry development.