Investigation of the physiological basis of the rind disorder oleocellosis in Washington navel orange (*Citrus sinensis* [L.] Osbeck)

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Abstract

Oleocellosis is a rind disorder of citrus fruit, which produces an unattractive surface blemish, and causes significant economic losses to the Australian citrus industry. It is caused by phytotoxic oils released from oil glands located in the rind, as a result of mechanical damage. In this study, microscopy investigations into the oil glands, localisation of the rind oils and the development of oleocellosis have been carried out in Washington navel orange fruit (*Citrus sinensis* [L.] Osbeck).

Changes in the structure, size and the number of oil glands located in the rind were assessed in the developing fruit from pre-anthesis to fruit maturity. Gland initiation was restricted to early fruit development, but glands continued to develop and reached maturity by fruit size 30 to 50 mm diameter. Mature glands continued to enlarge with fruit growth, but their final size and form varied within each fruit. Mature fruit had between 8,000 and 12,000 oil glands. Glands were found to develop from a cluster of cells adjacent to the fruit epidermis, into a structure consisting of a central cavity surrounded by several layers of epithelial cells. All glands were joined to the fruit epidermis by a 'stalk'. Gland cavity formation appeared to involve schizogeny.

Methods of tissue preparation were investigated in an effort to retain the essential oil contained within the glands. The modification of chemical fixation methods for improved rind oil retention gave limited success based on light microscopy observations. However, the examination of cryofixed material with scanning electron microscopy and fresh tissue with multi-photon microscopy were promising techniques for oil visualisation in orange rind tissue.

Oleocellosis was artificially induced in mature fruit under controlled conditions. This was achieved by mechanically damaging the fruit or applying rind oils to the fruit surface. Based on a range of criteria, penetrometer damage and d-limonene treatment were chosen as the optimal methods for inducing oleocellosis. These

methods were considered to simulate the two forms of naturally occurring oleocellosis; namely gland rupture and oil transfer between fruit.

Following induction, oleocellosis development was examined using a detailed time course assessment of surface symptoms and microscopic rind damage. Based on these observations, the events leading to oleocellosis blemish formation were proposed. Mechanical damage resulted in rupture of the epidermis above the glands and release of oil to the fruit surface. Surface oil appeared to infiltrate the rind via the cuticle, and via the ruptured epidermis in injured fruit. Once in the rind, the phytotoxic oils caused rapid cell content degeneration and cell collapse, with early stages of ultrastructural damage detected within six hours of induction. The resulting blemish, which was characterised by rind collapse and darkening, developed substantially within three days and was attributed to the sub-surface rind damage.