

**INTERNALIZATION OF *ESCHERICHIA COLI* IN APPLES UNDER FIELD
AND LABORATORY CONDITIONS**

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ABSTRACT

The main objective of this project is to gain an understanding of the internalization of *Escherichia coli* in the tissues of apples. This broad statement includes the rate of internalization in young versus mature apples as well as injured versus non-injured apples. Five apple varieties, Redfree, Red Delicious, Golden Delicious, Rome Beauty and York Imperial, were used to compare differences and similarities in structure and ability to internalize the pathogen. Both the surrogate species, *E. coli* ATCC 25922, and the pathogen, *E. coli* O157:H7, were used for field and lab studies, respectively.

Internalization of *E. coli* in apples under natural environmental conditions was addressed in the first study using a controlled outdoor setting. *Escherichia coli* species (ATCC 25922) was used as an alternative to the pathogenic species. The bacterial culture was applied to topsoil and spread evenly on a 6x6-foot area. Red Delicious, Golden Delicious, and Rome Beauty apples were placed randomly on the soil much like a drop or windfall apple. The position was noted as to whether the apple fell calyx up, down or on its side. Apples were examined for the presence of *E. coli* and sampled on days 1, 3, 8, and 10. Skin, flesh, inner, and outer core samples were plated on MacConkey agar supplemented with cycloheximide and MUG to ease in identification. *Escherichia coli* was found in the inner core and flesh samples of all apple varieties, indicating the potential for infiltration by the organism outside laboratory conditions.

The second study determined the rate of internalization in immature apples. Redfree was used in a long-term study in which individual apples were spray inoculated at the beginning of the growing season with *E. coli* ATCC 25922 at 10^4 cfu/apple. The

apples were picked on days 1, 30 and 60, and sectioned into skin, flesh, inner and outer cores. The remaining four apples species were used in an intensive, two-week study.

In the long-term study, apples were inoculated two weeks prior to harvest and picked every other day until harvest. The surrogate *E. coli* was not found in the apples after day 1. Other coliforms, such as *E. vulneris*, *Klebsiella pneumoniae* and *Kl. ozaenae* were present in each pick. The two-week study showed higher rates of internalization in Red and Golden Delicious than in Rome and York, with the *E. coli* present in all four sections of the apples. Red Delicious apples showed a trend of increasing counts of bacteria over the two-week period with initial counts ranging from less than one cfu/ml to final counts as high as 2.64 ± 1.90 log cfu/ml. Again *Klebsiella* species and *E. vulneris* were found in the apples.

Microscopy was used for imaging of the apples tissues. Morphological differences were found in the skin, where lenticel presence or absence may affect internalization. Differences were also shown in the flesh where cell wall thickness was shown to vary depending on variety. Imaging thick sections of skin showed cuticle cracks and thickness, which also vary depending on the apple variety.

This study indicates that internalization occurs at a high degree in drop apples and to a limited extent in tree apples. However, with the low infective dose required for illness, it is necessary to instate strict regulations to ensure safety. The most effective treatment involves the inclusion of a five-log reduction of the target organism, *E. coli* O157:H7. This reduction can be obtained through one step or the combination of two or more steps.

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