

**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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TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
INTRODUCTION.....	1
SECTION I: REVIEW OF LITERATURE.....	3
A. <i>Escherichia coli</i> Species.....	3
1. General Characteristics.....	3
2. <i>Escherichia coli</i> O157:H7 Characteristics.....	4
3. <i>Escherichia coli</i> O157:H7 Reservoirs and Transmission.....	4
4. Illness and Pathogenicity.....	5
5. Acid Resistance.....	6
6. Foodborne Outbreaks Associated with <i>Escherichia coli</i> O157:H7.....	7
7. Factors Affecting Growth in Apples an Apple Products.....	8
B. Apples.....	9
1. General.....	9
2. Development of the Apple from the Flower.....	10
i. Seed Development.....	10
ii. Carpel Development.....	11
iii. Flesh Development.....	11
iv. The Mature Apple.....	12
3. Chemical and Physiological Changes in the Apple throughout Development.....	13
i. Minerals.....	13
ii. Fatty Materials.....	13
iii. Enzymes.....	13
4. Microbiology of Apples.....	14
5. Cultivars of Apples.....	14
i. Red Delicious.....	15
ii. Golden Delicious.....	16

iii. Rome Beauty.....	16
iv. York Imperial.....	17
C. Electron Microscopy.....	17
1. Transmission Electron Microscopy.....	17
2. Laser Scanning Confocal Microscopy.....	18
3. Using Green Fluorescent Protein as a Marker.....	19
D. Contamination Issues in Apples and Apple Products.....	20
1. Regulation of Juice Products.....	20
2. Sources of Apple Contamination.....	22
3. Mechanisms of Internalization.....	23
4. Preventing Contamination in Apples.....	25
REFERENCES.....	27

SECTION II: INTERNALIZATION OF <i>ESCHERICHIA COLI</i> IN APPLES UNDER NATURAL CONDITIONS.....	32
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TITLE PAGE.....	33
ABSTRACT.....	34
INTRODUCTION.....	35
METHODS AND MATERIALS.....	37
Apples.....	37
Outdoor Apple Pen.....	37
Inoculum.....	37
Apple Sample Preparation.....	38
Electron Microscopy.....	38
RESULTS.....	39
Microbial Analysis.....	39
Electron Microscopy.....	40
DISCUSSION.....	40
ACKNOWLEDGEMENTS.....	42
REFERENCES.....	42

SECTION III: INTERNALIZATION OF <i>ESCHERICHIA COLI</i> IN FIELD AND LABORATORY CONDITIONS.....	49
--	----

TITLE PAGE.....	50
ABSTRACT.....	51
INTRODUCTION.....	52
METHODS AND MATERIALS.....	54
A. Field Studies.....	54

Preparation of Field Study Inoculum.....	54
Inoculation of Field Study Apples.....	55
Analysis and Enumeration of Field Study Apples.....	55
Experimental Design and Statistical Analysis.....	56
B. Microscopy.....	56
Laser Scanning Confocal Microscopy.....	56
Preparation of Inoculum.....	57
Inoculation and Examination of Apples.....	57
Experimental Design and Statistical Analysis.....	58
Transmission Electron Microscopy.....	58
General Microscopy.....	58
RESULTS	59
A. Field Studies.....	59
Long-term Studies.....	59
Short-term Studies.....	59
B. Microscopy.....	61
Laser Scanning Confocal Microscopy.....	61
Transmission Electron Microscopy.....	61
General Microscopy.....	62
DISCUSSION.....	62
ACKNOWLEDGEMENTS.....	64
REFERENCES.....	65
VITAE.....	81

LIST OF TABLES

Chapter 2

- Table 1. Location of *E. coli* ATCC 25922 infiltration in the inner and outer cores of different apple varieties.....45
- Table 2. Location of *E. coli* ATCC 25922 infiltration in the skin and flesh of different apple varieties.....46

Chapter 3

- Table 1. Location of *E. coli* ATCC 25922 infiltration in the skin, flesh, inner and outer cores of immature Redfree apples, treated June 5 and harvested through August.....67
- Table 2. *E. coli* ATCC 25922 infiltration in the skin, flesh, inner and outer cores of mature Red Delicious and Golden Delicious apples, spray inoculated outdoors August 28 and September 6 respectively.....68
- Table 3. Location of *E. coli* ATCC 25922 infiltration in the skin, flesh, inner and outer core of York Imperial and Rome Beauty apples, spray inoculated outdoors September 25 and October 1, respectively.....69
- Table 4. Location of *E. coli* ATCC 25922 infiltration in the skin, flesh, inner and outer cores of Red Delicious apples in a controlled indoor setting.....70
- Table 5. Location of *E. coli* ATCC 25922 and infiltration in the skin, flesh, inner and outer cores of mature Red Delicious apples in a controlled indoor setting using dip inoculation after a three day incubation.....71

LIST OF FIGURES

Chapter 1

Figure 1. Light Scattering Pattern Produced by Transmission Electron Microscopes....18

Chapter 2

Figure 1. Lenticels from Rome Beauty (RB), Red Delicious (RD), and Golden Delicious (GD) skin samples are indicated by the black arrows.....47

Figure 2. Cell walls of Rome Beauty (RB), Red Delicious (RD) and Golden Delicious (GD).....48

Chapter 3

Figure 1. Preliminary Growth Studies of *Escherichia coli* ATCC 25922 held at room temperature in sterile spring water for a three-day period.....72

Figure 2. *E. coli* counts in the skin, flesh and inner core of the Red Delicious over the two-week period of the short-term study.73

Figure 3. The rate of internalization in the outer core of the short-term Red Delicious apple is related to the day.....74

Figure 4. The proportion of Golden Delicious apples with *E. coli* present in the skin and in the inner core over the two-week period of the short-term75

Figure 5. Counts in the flesh, skin and inner core of the indoor Red Delicious apple over the two-week period of the short-term study.....76

Figure 6. Counts in the outer core and the inner core of the indoor Red Delicious apple.....77

Figure 7. Lenticels on mature skin samples, Golden Delicious (A), York Imperial (B) and Red Delicious (C) at a magnification of 25,000X, are indicated by arrows...78

Figure 8. Cell wall and hemicellulose of mature Golden Delicious (A), York Imperial (B) and Red Delicious(C) at 25,000X magnification.....79

Figure 9. Cuticle of mature Golden Delicious (A), York (B) and Red Delicious (C) at 300X magnification.....80