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Plant Lesions Promote the Rapid Multiplication of *Escherichia coli* O157:H7 on Postharvest Lettuce

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ABSTRACT

Several outbreaks of *Escherichia coli* O157:H7 infections have been associated with minimally processed leafy vegetables in the United States. Harvesting and processing cause plant tissue damage. In order to assess the role of plant tissue damage in the contamination of leafy greens with *E. coli* O157:H7, the effect of mechanical, physiological, and plant disease-induced lesions on the growth of this pathogen on postharvest romaine lettuce was investigated. Within only 4 h after inoculation, the population sizes of *E. coli* O157:H7 increased 4.0-, 4.5-, and 11.0-fold on lettuce leaves that were mechanically bruised, cut into large pieces, and shredded into multiple pieces, respectively. During the same time, *E. coli* O157:H7 population sizes increased only twofold on leaves that were left intact after harvest. Also, the population size of *E. coli* O157:H7 was 27 times greater on young leaves affected by soft rot due to infection by *Erwinia chrysanthemi* than on healthy middle-aged leaves. Confocal microscopy revealed that leaf tip burn lesions, which are caused by a common physiological disorder of lettuce, harbored dense populations of *E. coli* O157:H7 cells both internally and externally. Investigation of the colonization of cut lettuce stems by *E. coli* O157:H7 showed that the pathogen grew 11-fold over 4 h of incubation after its inoculation onto the stems, from which large amounts of latex were released. The results of this study indicate that plant tissue damage of various types can promote significant multiplication of *E. coli* O157:H7 over a short time and suggest that harvesting and processing are critical control points in the prevention or reduction of *E. coli* O157:H7 contamination of lettuce.

Plant growth conditions. Romaine lettuce plants (*Lactuca sativa* cv. Parris Island) were used throughout these studies. The plants were grown to mature heads in Sunshine Mix 1 (Sun Gro Horticulture Distribution Inc., Bellevue, WA) in a greenhouse with a 16-h photoperiod and day and night temperatures of 24°C and 18°C, respectively, before the leaves were harvested for the experiments. The plants were fertilized weekly, starting at 2 weeks after emergence, with 1 mg of NKP (Spectrum Brands, Inc., Atlanta, GA) (20:20:20) per plant. Romaine lettuce heads that were grown and packed in boxes in the field were purchased directly from the distributor and used to test the effect of mechanical lesions on the growth of *E. coli* O157:H7.

Stem inoculations. The stem of mature heads of lettuce plants grown in the greenhouse was cut 2 cm above the soil line. A disc 1 cm in thickness was cut from the stem and placed on wet filter paper in a petri dish (Fig. 1). Each replicate disc came from a different plant. Three discs were placed per petri dish. A 50-μl drop of a 10^4 cells/ml of *E. coli* O157:H7 suspension prepared as described above was spread in the latex drop oozing from the disc. The replicate petri dishes were covered, sealed with Parafilm M (American National Can, Chicago, IL), and incubated at 28°C.

Open in a separate window

FIG. 1.

Photograph of a stem disc cut from a mature romaine lettuce plant at harvest. The stem released a large quantity of latex (black arrow) from the laticifers upon wounding of the stem tissue. Stem discs such as this one were inoculated with *E. coli* O157:H7 and placed in a petri dish for incubation at 28°C and measurement of bacterial growth in the latex and cut surface.

Leaf inoculations.

respectively. Plates with *E. coli* O157:H7 were incubated at 37°C for 24 h, whereas plates with *E. chrysanthemi* were incubated at 30°C for 48 h. Population sizes on the leaves and stem discs were assessed by plate counts.

Microscopy. One-centimeter-thick leaf discs were sampled from tip burn lesions. The discs were mounted in AquaPoly/mount (Polysciences, Warrington, PA). The GFP signal from the bacteria and the red autofluorescence of the plant cells were visualized using a Leica TCS-NT confocal microscope (Leica Microsystems, Wetzlar, Germany) with emission filter sets BP525/50 and LP590, respectively.

Statistical analyses. All experiments were replicated at least twice. All statistical analyses were performed with Prism version 3.0 software (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSION

Growth of *E. coli* O157:H7 on cut lettuce stems. At harvest, lettuce plants are cut at the base of the stem. Therefore, the fate of *E. coli* O157:H7 on the cut stem after a potential contamination event at harvest was investigated in this study. Lettuce is one of few edible crops that produce latex (14, 23). Upon cutting of lettuce stems, a large quantity of latex is released from the laticifers onto the cut surface (Fig. 1). After harvest, the latex on the stems rapidly changes color from white to brown, dries, and can be transferred to other parts of the lettuce by rubbing of the stem of one head against the leaves of another. Browning and drying of the latex was also observed within the first hour after harvest in this study despite the incubation of the stem discs under humid conditions.

As early as 2 h and 4 h after its inoculation onto lettuce stem discs, population sizes of *E. coli* O157:H7 had increased 5.6- and 11.1-fold (Table 1). By 22 h of incubation, the *E. coli* O157:H7 population size on the stem discs had increased 20,091-fold, suggesting that the surface of cut lettuce stems holds large quantities of substrates that allow for the multiplication of *E. coli* O157:H7. In addition to the metabolites that leaked from the plant cells due to cutting, the presence of sugars in the lettuce latex (14) may have promoted the growth of *E. coli* O157:H7 on the cut stems. Indeed, after its inoculation into latex that was collected from lettuce stems and diluted 100-fold in distilled water, *E. coli* O157:H7 cells grew 10-fold within 12 h at 28°C (data not shown). Although lettuce latex contains lettucenin A (23), a phytoalexin that has a role in the resistance of lettuce tissue to infection by bacterial plant pathogens such as *Pseudomonas syringae* pv. *phaseolicola* (2), this apparently did not prevent growth of *E. coli* O157:H7 on the cut stems.

DR. MIKE Doyle

ANIMALS, FOOD & DRINK, PLANTS

Why Lettuce Keeps Making Us Sick

JUL 16, 2013

Andre Gallant

When it comes to beef and poultry, pathogens like salmonella and E. coli O157:H7 can be killed by proper foodborne fate is sealed in, literally, on the farm during harvest and processing.

During harvest, workers core lettuce in the field, often with a knife soiled by pathogen-laden dirt. The plant then produces a milky latex that essentially traps any present pathogens in the plant. And as Michael Doyle, director of the University of Georgia's Center for Food Safety, explained, no amount of water can kill microorganisms. The good news, though, is that the Food Safety Modernization Act, once it eventually goes into practice, will usher in a new era of food safety on the farm, requiring produce growers, just the bigger ones, really, to enact major changes to how vegetables are grown, harvested and stored.

We asked Michael Doyle to talk a bit more about what's happening in the fields and what, if anything, is going to change.

Modern Farmer: Can you explain the interaction between lettuce and microorganisms during harvesting?

A food production wiki for public health professionals

Romaine Lettuce

Last Updated: 2 weeks ago

Contents

extensively in organic production. In minor production areas, transplants may be preferred due to the short growing season.

Several types of irrigation (sprinkler, drip, and furrow) are used to provide continuous moisture, which allows for maximum yields and quality. Initially, sprinkler irrigation (2 to 4 in) is used prior to seeding to soften the soil for seedbed preparation. Then every two to three days, water is applied to seeded soil or transplants until seeds emerge or transplants have been established (usually 6 to 10 days after transplanting). Irrigation continues for the remainder of the growth cycle, with the highest use of water occurring during the last month of crop growth. During this time, surface drip irrigation with drip tape is often installed and utilized as it permits growers to water frequently during this rapid vegetative growth phase. This type of irrigation is also advantageous because romaine lettuce is shallow-rooted, and drip irrigation, rather than sprinkler overhead irrigation, is more effective at delivering water directly to the roots. Therefore, less water is required (12 to 18 in for drip compared to 18 to 24 in for spray irrigation). Drip irrigation also facilitates weekly fertigation with low rates of fertilizer as opposed to having to apply side-dressings of nitrogen close to the romaine lettuce roots (applied typically two to three weeks after seeding and thinning has taken place). When used, drip tape is installed between rows and typically retrieved before harvesting so that it may be reused for subsequent crops. Furrow irrigation, the least efficient in terms of water usage (24 to 30 in), is used primarily in Arizona, which has an abundant water source from the Colorado River and is a much cheaper option for producer there.

Several other growing practices may be used to extend the growing season of romaine lettuce, including growing plants in high tunnels, greenhouses, or in hydroponic systems. In Florida, the use of hydroponic systems has grown rapidly. The predominant hydroponic design uses plastic or other lightweight channels, gutters, or tubes that hold multiple transplants. A thin film of nutrient solution trickles over the bare roots of each plant. The whole system is at a sloped angle, enabling catchment of the unused nutrient solution, which is then filtered or aerated and recycled back to a reservoir for reuse. However, Hydroponics remains a very expensive option and makes up a very small portion of commercial growers.

Harvesting and Postharvest

Historically, romaine lettuce has been hand harvested as whole plants; however, small growers selling bagged greens may choose to harvest the crop as individual leaves. Typically, the time for romaine lettuce to reach market maturity will depend on growing conditions and variety. Waiting too long to harvest can result in bolting. Once the romaine head is cut at the base, the head is trimmed of loose, discolored, damaged, diseased, and soiled leaves. The heads are then placed on a conveyor belt where they are sprayed with a chlorinated water solution (generally 200 ppm) before being packed into 24 count (head) cartons.

Hand harvesting of lettuce requires a large amount of human capital. For example, in Arizona, more than 45,000 legal guest workers from Mexico are commuted across the border into Yuma every day for the purposes of harvesting up to 1 million boxes of lettuce each day. Automated, mechanized romaine harvesters, which use a water knife to cleanly cut the heads in the field, are now being used in some commercial operations.

Romaine lettuce heads have moderate respiration rates, which are generally higher than rates for iceberg lettuce. Therefore, it is even more critical that the product is quickly cooled to extend its shelf-life. Cartons of romaine lettuce are shipped to a facility where field heat is generally removed through vacuum cooling. Once these operations are performed, romaine typically has a storage life of two to three weeks if stored at the proper temperature (0 to 5°C) and relative humidity (95%). Although low oxygen atmospheres will reduce respiration, atmospheres containing carbon dioxide are not generally beneficial to intact heads. In contrast, cut romaine lettuce is commonly packaged in low oxygen (<1%) and high carbon dioxide (7 to 10%) atmospheres because these conditions control browning on cut surfaces.

Pre-Harvest

Cross Contamination

Ramsay HIGHLANDER INC

CUSTOM HARVESTING AIDS to Increase Productivity

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Products

Mechanical Harvesters

- Spinach/Spring Mix
- Spinach/Spring Mix BL Mini
- Romaine/Green Leaf

Harvesting Aids

- 3-point Conveyor Systems
- Self-propelled Bulk Loaders
- Field Pack Harvesting Aids

Harvesting Accessories

- Software Technology
- Accessories

Romaine/Green Leaf Lettuce Water Jet Mechanical Harvester

Water Jet Product Details

Ramsay Highlander's newest product launch, the Mechanical Harvester. Designed to harvest Romaine, Green Leaf and a version for Iceberg and many other commodities. Utilizing Ramsay's patented **Water Jet cutting method** this harvester increases yield and product shelf life while reducing labor. Having an individual high pressure water stream for every seed line allows you to independently adjust cut height for uneven beds. The HP nozzle carriers are easily reconfigured to accommodate all bed widths and seed line spacing. The Mechanical Harvester also helps eliminate trash leaves before reaching the sorting/packing belt. The rear of the machine can be configured for totes or bins to meet your requirements. It has a capability to harvest 12,000 pounds of Romaine per hour into totes and up to 24,000 per hour in a bulk loading version.

This harvesting method is the way of the future because of it's increased yield, it's natural sanitary cutting method, and it's ability to negotiate uneven beds. It consumes less than 1.5 GPM of water when harvesting 6 seed lines and has a 325 Gal. reservoir with quick fill attachments. If you have unpredictable field conditions, this water jet harvester provides a labor saving harvesting solution.

See the **Water Jet Mechanical Harvester Video**

Field Crops

Romaine Lettuce, Green Leaf Lettuce, Iceberg Lettuce

Features

- Stainless Steel
- Track Drive
- 175hp John Deere Engine
- 120" wide for transport
- QC Deck
- Trash Separation System
- Tote or Bin Configuration
- Maneuverability

Options

- Canopy
- Lighting System
- Pressure Washer
- Water System
- Top and Tail Attachment
- Copper Ion System

Video Gallery

Water Jet Harvester

View the Flash Video
10.6 MB - 3:32 sec
(or view on YouTube)

Get Adobe Flash Player

You will need Flash Player to view videos.
Download now.

CROSS contamination

Romaine/Green Leaf Lettuce Headrazor Mechanical Harvester

Storage and Cooling Conditions

Lettuce is best maintained as close to 0°C as possible. Because of lettuce's large water content, a high humidity level is preferable. Lettuce is highly sensitive to ethylene and will decay if not isolated from high ethylene-producing produce in storage. Figure 2 indicates ideal storage conditions:

Produce	Optimal Storage Temp., °C	Optimal Humidity (%)	Cooling with top ice acceptable	Cooling with water sprinkle acceptable	Ethylene Production	Ethylene Sensitivity to	Storage Life
Lettuce	0	98-100	No	Yes	No	Yes	2-3 Weeks

Table 2. Storage and Cooling Conditions for Lettuce (Fellow, 2000)

Good Agriculture Practices (FDA, 2006)

- Develop and implement procedures for preventing pest infestation in irrigation pipe and drip tape.
- Ensure that water used in all pre- and post- harvest applications meets microbial water standards. This includes water used for hydrovac cooling.
- Any cooling equipment used on lettuce should be cleaned and sanitized regularly.
- Never use raw animal manure on or near lettuce.
- Chlorine is insufficient to kill pathogens in lettuce latex after cutting and coring. A stronger FDA-approved sanitizing agent should be used.

Pathogenic Behavior in Commodity

Typical cleaning practices are incapable of completely eliminating bacteria from surface-contaminated produce. The only way to eliminate contamination is to prevent pathogens from coming into contact with lettuce during the entire farm-to-fork process. Pathogens can easily be splashed onto plants from soil or transferred by human or animal contact. Dried manure can also be wind-blown onto plants, and *Salmonella* has been shown to be resistant to drying. Noroviruses are another concern for lettuce. Noroviruses are the most common foodborne disease and are often found in lettuce. Norovirus is significantly smaller than bacteria, and has been shown to be capable of transferring up into the lettuce plant from the roots.

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This food safety factsheet can be downloaded at http://www.wku.edu/agriculture/index.php