ROUTES OF CONTAMINATION

ATTACHMENT AND RECOVERY OF *ESCHERICHIA COLI* O157:H7 AND A NON-PATHOGENIC SURROGATE FROM ROMAINE LETTUCE AFTER CONTACT WITH CONTAMINATED ICE

(J. Kim and M. Harrison)

Ice, possibly contaminated with *E. coli* O157:H7, can be used to chill romaine lettuce and maintain relative humidity during transportation. Contamination of lettuce is of concern since it is usually consumed raw or minimally processed. The potential for *E. coli* O157:H7 contamination of romaine lettuce with either ice contaminated with the pathogen or by transfer from lettuce surfaces via melting ice was determined. In order to evaluate pathogen transfer by these means in actual commercial facilities, the use of non-pathogenic surrogates is needed. A non-pathogenic *E. coli* strain was selected and compared with *E. coli* O157:H7 to determine differences and similarities in attachment to and recovery from romaine lettuce in contact with contaminated ice. *E. coli* O157:H7 distributes onto other produce layers in shipping containers due to melted ice made of contaminated water and transfers from contaminated to uncontaminated surfaces. Based on cryotolerance and cell surface characteristics, *E. coli* ATCC 25922 is a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recovery from chilled produce.

CONTAMINATION AND POTENTIAL INTERNALIZATION OF *ESCHERICHIA COLI* O157:H7 IN PRE-HARVEST ICEBERG LETTUCE (*LACTUCA SATIVA L.*)

(G. Zhang, L. Ma, L.R. Beuchat, M.C. Erickson, V.H. Phelan, and M.P. Doyle)

The ability of foodborne pathogens to internalize within lettuce, especially under growing conditions, is an important unanswered question in need of elucidation for risk analysis and intervention purposes. The objectives of this study were (1) to determine the effect of inoculation sites (abaxial vs adaxial leaf surfaces) on survival and internalization of *E. coli* O157:H7 in lettuce; and (2) to evaluate the vulnerability of lettuce at different ages to *E. coli* O157:H7.

Iceberg lettuce (*Lactuca sativa L.*) was grown in sandy soil in an envirotron at 23°C during the day and 7°C at night. A 5-strain mixture of GFP-labeled *E. coli* O157:H7 at 10^6 CFU/ml in water and cow manure extract was used as inoculum. Plants were inoculated on abaxial and adaxial sides of leaf surfaces at 3, 30, and 60 days after transplantation and sampled 2 to 3 times for each inoculation treatment. At each sampling time, *E. coli* O157:H7 in soil and in/on shoots and roots were analyzed. For surface-sterilization, leaves and roots were dipped in 80% ethanol for 10 s, followed by immersion in 0.1% $HgCl_2$ for 10 min.

Twenty-five days after inoculation, 2 of 12 samples were *E. coli* O157:H7-positive on inoculated leaves. No *E. coli* O157:H7 was detected on inoculated leaves at 54 days. All surface-sterilized root and leaf samples were negative for *E. coli* O157:H7 regardless of plant age at inoculation, sampling time, or abaxial- or adaxial-side inoculation. Substantially more lettuce leaves inoculated on the abaxial side were *E. coli* O157:H7-positive after 3 to 25 days than those leaves inoculated on the adaxial side.

Internalization of the *E. coli* O157:H7 in iceberg lettuce by leaf inoculation did not occur. Age of lettuce plants did not affect internalization of *E. coli* O157:H7 in lettuce. Inoculated *E. coli* O157:H7 survived longer on the abaxial side of the leaves than on the adaxial side.

CONTAMINATION AND POTENTIAL INTERNALIZATION OF *ESCHERICHIA COLI* O157:H7 IN LETTUCE (*LACTUCA SATIVA* L.) BY SOIL INOCULATION

(G. Zhang, L. Ma, L.R. Beuchat, M.C. Erickson, V.H. Phelan, and M.P. Doyle)

Understanding whether internalization of foodborne pathogens occurs through plant roots will be helpful in conducting risk assessments and developing effective interventions to reduce pathogen contamination in produce. The objectives of this work were (1) to determine if internalization of *E. coli* O157:H7 through lettuce roots occurs; and (2) to determine if differences exist among *E. coli* O157:H7 isolates and lettuce types regarding *E. coli* O157:H7 internalization, survival and growth in and on lettuce plants.

Iceberg, Romaine and leaf lettuces were grown in sandy soil in an envirotron using two temperature regimes. Soil was inoculated with 5 GFP-labeled *E. coli* O157:H7 isolates individually at 10^6 or 10^3 CFU/g of soil when

lettuce seedlings were transplanted. Lettuce plants were sampled 2 to 3 times after transplantation and assayed for *E. coli* O157:H7 in soil and in/on shoots and roots. For surface-sterilization, leaves and roots were dipped in 80% ethanol for 10 s, followed by immersion in 0.1% $HgCl_2$ for 10 min.

Results revealed that surface-sterilized leaf and root samples were negative (except for 2 root samples) for *E. coli* O157:H7. Seventeen days after transplantation and inoculation, most leaf surfaces were positive for *E. coli* O157:H7 which was likely due to cross-contamination from the inoculated soil. The 26-, 45- and 60-day samplings revealed no *E. coli* O157:H7 on leaf surfaces. Some soil and rhizosphere samples were positive for *E. coli* O157:H7 at 60 days when the trials were terminated.

In conclusion, internalization of *E. coli* O157:H7 in lettuce did not occur through the roots; however, the pathogen could survive in soil for at least 60 days. There were no differences among *E. coli* O157:H7 isolates or lettuce types with regard to *E. coli* O157:H7 internalization in lettuce.

PRE-HARVEST FACTORS AFFECTING INTERNALIZATION OF ZOONOTIC PATHOGENS INTO LETTUCE

(M.C. Erickson, J. Liao, A. Payton, C. Webb, L. Ma, G. Zhang, M. Doyle, and L.R. Beuchat)

In the past two decades, the fresh fruit and vegetable industry has rapidly evolved and contributed to increased retail and food-service sales. Accompanying this growth has been an increasing number of outbreaks associated with fresh produce consumption that has often been traced back to the farm. Potential pre-harvest vehicles for contamination of vegetables include soil amendments (manure or improperly-composted manure) or contaminated irrigation or runoff water. Based on laboratory studies, however, both surface and internalized contamination occurred when seeds or seedlings were exposed to contaminated soil or water solutions. Whether internalization occurred in older plants and the fate of any internalized populations was one of the objectives of this study.

Differences in the robustness of plant defense mechanisms that target bacterial extracellular components for subsequent subcellular compartmentalization and degradation have been suggested as one factor affecting internalized pathogen populations. Since plant stress associated with drought conditions could affect plant defensive activities, the level of internalization of zoonotic pathogens could, in turn, also be affected. Another factor that is likely to affect internalization of zoonotic pathogens is the level of indigenous microorganisms in the soil environment. Since the abundance of an indigenous population is dependent on the relative availability of nutrients, internalization of zoonotic pathogens by plants could, in turn, be affected by the level of fertility in the soil. A second objective of this study therefore addressed both the influence of plant stress and soil fertility levels on internalization of zoonotic pathogens by lettuce plants.

Green leaf lettuce (variety Two star) was grown in pots using either 0:5, 1:5 or 2:5 manure compost:top soil mixtures. Pots were held in an envirotron at 20°C during the day and 7°C at night. An inoculum mixture of green-fluorescent protein (gfp)-labeled *Escherichia coli* O157:H7 isolates or an inoculum mixture of gfp-labeled *Salmonella* spp. was prepared and added to water to give concentrations of 10^3 or 10^6 CFU/ml. Contaminated water was applied to the soil of 3- or 33-day post-transplanted plants (30-50 ml/plant) and a portion of those plants were sampled 3 days later and at 60-days post-transplantation. For a sub-group of plants exposed at 33-days post-transplantation, a reduced watering rate was applied for 2-3 weeks prior to the contamination event. With all plants, a physical barrier separated leaves and soil to prevent direct transfer of pathogens from soil to leaves. Leaves were analyzed separately from washed roots and both surface and internalized populations were enumerated for these samples. Using an ethanol and mercury chloride wash, surface sterilization of samples preceded enumeration of internalized populations.

Pre-harvest internalization of *Escherichia coli* O157:H7 or *Salmonella* spp. into roots or leaves of green leafy lettuce cultivated in a growth chamber did not occur when plants were watered with a contaminated water source. Pathogen internalization was not affected by the level of soil fertility. A 2-week period of reduced watering prior to the contamination event also did not induce internalization of pathogens. The absence of internalized populations is of merit as post-harvest interventions need only target surface contamination.