International Journal of Food Microbiology 153 (2012) 345–350

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Assessment of *Escherichia coli* O157:H7 transference from soil to iceberg lettuce via a contaminated field coring harvesting knife

Yang Yang [a,b], Yaguang Luo [a,*], Patricia Millner [a], Ellen Turner [a], Hao Feng [b]

[a] Environmental Microbial and Food Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Building 002, Room 117, 10300 Baltimore Avenue, Beltsville, MD 20705, United States
[b] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States

ARTICLE INFO

Article history:
Received 21 March 2011
Received in revised form 23 August 2011
Accepted 27 November 2011
Available online 2 December 2011

Keywords:
Leafy green
E. coli O157:H7
Coring knife
Soil

ABSTRACT

The potential for lettuce field-coring harvesting knives to cross-contaminate lettuce heads with pathogens was evaluated. Rings and blades of the harvest knives artificially contaminated with *Escherichia coli* O157:H7 (EHEC), were used to core three successive heads of iceberg lettuce. The coring rings and blades were inoculated by dipping into soils containing EHEC at concentration ranges of 1–10^5 MPN/g soil. Factors that influenced EHEC transference from soil to iceberg lettuce via contaminated coring knife blade, included water content (WC) of clay and sandy soils, EHEC concentration, and degree of blade contact (stem, medium, and heavy) with edible tissue. High moisture content clay soil was positively associated with high pathogen transference. No EHEC were detected on any cut heads when clay soil contaminated with 10^5 MPN/g EHEC had WC of 20% or less, or when the knife blade was dipped into sandy soil contaminated with EHEC at the same level, regardless of percent WC. The extent to which the harvesting knife blade cut across edible lettuce tissues was also an important factor in the amount of pathogen transference that occurred. EHEC were detectable on first and second sequentially cut lettuce heads when medium-contact was made between knife blade and edible tissues and on all three sequentially cut lettuce heads using the heavy-contact cutting scenario, when the blade was contaminated with 10^4 cfu/g EHEC in clay soil (25% WC). However, when the blade, contaminated at the same soil EHEC level, was used to cut only the stem and had no contact with the edible portion of the lettuce head, no pathogen transference was detected. Under the current CIF harvesting practice, the cutting blade has a higher potential than the coring ring to be contaminated by the soil, but less opportunity to transfer pathogens to harvested lettuce. However, once contaminated, the coring ring has much higher potential than the blade to transfer pathogens to the harvested lettuce.

Published by Elsevier B.V.

1. Introduction

Packaged fresh-cut lettuce has gained broad market acceptance for its convenience and fresh nutritional value. However, lettuce has increasingly been associated with foodborne illness outbreaks (Berger et al., 2010; CDHS, 2007; Cooley et al., 2007; Lynch et al., 2009; Doyle and Erickson, 2008). *Escherichia coli* O157:H7 is the most common bacterial etiologic agent in outbreaks associated with lettuce and other leafy greens, and is of particular public health concern because of the potential severity of the associated acute gastrointestinal illness as well as the chronic sequelae that can result. Although contamination of lettuce can occur at any point in the farm-to-plate continuum, exposure to irrigation water, soil, soil amendments, animals, handling by field workers and equipment make the field production stage particularly high risk for *E. coli* O157:H7 contamination of lettuce and other leafy greens.

Lettuce trimming and coring-in-field (CIF) are relatively recent industry developments designed to increase processing plant production yields from traditional levels of 60–70% to nearly 100% by removing wrapper leaves and outer leaves in the field and harvesting only ready to process lettuce (Anon, 1996, 2001). This process significantly reduces shipping and waste disposal costs while maintaining the market quality of lettuce (Brown and Rizzo, 1999, 2001). The CIF practice has been widely adopted for harvesting lettuce destined for fresh-cut processing (Suslow et al., 2003; De Groot et al., 2008). During CIF, the outer/wrapper leaves are removed and only relatively clean inner leaves are harvested.

However, core removal requires additional human handling per head in the field and exposes the internal leaf tissues, increasing the risk of direct contamination, which is already high in field environments (FAO/WHO, 2008). Cut leaf tissues, such as those resulting from coring, provide a moist, nutrient rich environment especially conducive to direct and rapid infiltration, and pathogen attachment, growth and survival (Takeuchi et al., 2000). Barker-Reid et al. (2009) showed that E. coli had much greater persistence on damaged iceberg lettuce plant tissues than on undamaged plants.

* Corresponding author. Tel.: +1 301 504 6186; fax: +1 301 504 5107.
E-mail address: Yaguang.Luo@ars.usda.gov (Y. Luo).

0168-1605/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2011.11.024

Coring knives may be contaminated through direct contact with contaminated soil, plants, or workers' gloves or clothing, and thereby act as vehicles for sequential contamination of edible portions of harvested lettuce heads (Taormina et al., 2009). Studies have shown that a single contaminated coring knife could transfer *E. coli* O157:H7 to as many as 19 lettuce heads, depending on the initial contaminant density (McEvoy et al., 2009). However, limited data are available on pathogen transference at realistically low contamination densities for risk assessment under real world conditions. The purpose of this study was to determine the pathogen concentrations required for *E. coli* O157:H7 transference to the edible portions of lettuce and to identify the essential factors involved in pathogen transference from soil to lettuce via a contaminated coring knife, so that preventative measures can be taken.

2. Materials and methods

2.1. Plant materials

Iceberg lettuce (*Lactuca sativa* L.) was obtained from a local produce wholesale market (Jessup, MD), stored at 5 °C, and used within 24 h. Lettuce heads were selected for similar shape and size. Soiled and damaged outer lettuce leaves were removed and the stem was trimmed immediately prior to coring.

2.2. Escherichia coli O157:H7 strain

Escherichia coli O157:H7, strain CDC B6914, used throughout the experiment contains a plasmid which produces green fluorescent protein and confers ampicillin resistance (Fratamico et al., 1997). The strain was grown in tryptic soy broth (TSB, Becton Dickenson & Co., Sparks, MD) supplemented with 200 μg/ml ampicillin and incubated overnight at 37 °C. Cells were harvested by centrifugation for 5 min at 7500 rpm (5 °C), washed twice in sterile phosphate buffered saline (PBS) before re-suspension in PBS, and serially diluted to obtain the target initial concentration. The final actual inoculum density was determined by an 8-tube modified most probable number (MPN) method (Nou and Luo, 2010).

2.3. Coring ring inoculation and transference

Commercially used lettuce coring knives were obtained from Agricultural Supplies Inc. (Salinas, CA). The knife structure consists of a cutting blade at one end, a handle in the middle, and a coring ring at the other end (Fig. 1). Knives were autoclaved prior to use. Transference of *E. coli* O157:H7 from the ring end of the coring knife to the lettuce was investigated using two inoculation methods: liquid and soil inoculation.

2.3.1. Liquid inoculation

The outer edge of the coring ring (about 0.5 cm from cutting edge) was spread evenly by pipet with 20 μl of *E. coli* O157:H7 inoculum containing cell densities of 10^0–10^1, 10^1–10^2, 10^2–10^3, and 10^3–10^4 MPN/coring ring. The inoculum was allowed to partially air dry on the coring ring for approximately 30 s prior to the successive coring of three heads of iceberg lettuce. A negative control was included by pipetting 20 μl sterile PBS onto the edge of the coring ring instead of inoculum.

2.3.2. Soil inoculation

Organic soil, a Chualar loamy sand (fine-loamy, mixed, thermic Typic Argixerol) was obtained from a research farm (Salinas, CA) growing leafy greens. Soil was dried to constant weight by repeated, short-pulse (15 s) microwaving, and finally autoclaved (15 min, 121 C). For each treatment, 90 g of dried, autoclaved soil was spread uniformly over a rectangular tray and inoculated with 10 ml of various concentrations of *E. coli* O157:H7 by adding ten 125 μl droplets from each tip of an eight-channel pipette at 18 mm intervals over the soil surface. The soil was mixed thoroughly by gentle stirring with a sterile pipette and analysis of 1 g subsamples by a modified MPN method showed that cell density after mixing was uniform. The WC of each inoculated soil was verified by weighing out 2 g soil samples and measuring the weight loss after microwaving for 2 min. A total of five *E. coli* O157:H7 concentration ranges (10^0–10^5 MPN/g soil) were tested.

The ring end of an autoclaved coring knife was inoculated by insertion to a depth of 5 mm into soil contaminated at one of the five different *E. coli* O157:H7 concentrations for 1 min. The amount of attached soil was determined by subtracting the weight of the coring knife, before insertion from the weight, after insertion. The contaminated coring knife was then used to core three successive heads of lettuce. A negative control was included by coring lettuce heads with a coring knife dipped into an autoclaved, un-inoculated loamy sand soil sample at 10% WC.

2.4. Cutting blade inoculation and transference

2.4.1. E. coli O157:H7 inoculation and soil characteristics

Chualar loamy sand soil (with 25% coarse and very coarse sand particles, 1% organic matter, and alkaline pH) at one of three different water contents (WC) (5%, 10% and 15%) and clay soil (with 25% clay, 15% fine sand, and 2% organic matter also with alkaline pH). at one of five WC (10%, 15%, 20%, 25%. and 30%) were used for cutting blade studies. Clay soil was obtained from USDA-ARS leafy green fields in Salinas, CA. Clay soil was sieved (2 mm) after drying as previously described. The dried soils were autoclaved and portions of 475 g, 450 g, 425 g, 400 g, 375 g and 350 g were spread uniformly over rectangular trays. A multichannel pipette was used to evenly distribute 10 ml of 10^7 MPN/ml *E. coli* O157:H7 inoculum over the entire soil surface as described above, before thorough mixing. Each soil sample was brought to 500 g by adding 15 ml, 40 ml, 65 ml, 90 ml, 115 ml and 140 ml sterile PBS, respectively. The final *E. coli* O157:H7 concentration for all soils was 10^5 MPN/g of soil. The coring knife blade was then inoculated by insertion into the *E. coli* O157:H7 contaminated soil mixes following a procedure described by Taormina et al. (2009). Slight pressure was applied on the handle towards one side

Fig. 1. A typical, commercial lettuce field coring/harvesting knife with A (the cutting blade) and B (the coring ring).

of the flat blade surface while pulling the blade out of the soil. The blade was reinserted into soil, and removed while pressing towards the opposite side such that both sides contacted the soil equally. The amount of attached soil was determined by the same method used for the coring ring. The contaminated coring knife blade was then used to cut three successive heads of lettuce, followed by core removal using un-inoculated coring rings.

2.4.2. Evaluating the effect of cutting-blade contact scenarios on E. coli transference

Three different cutting-blade contact scenarios: stem-contact, medium-contact and heavy-contact, which reflect the range of cuts observed on commercially harvested heads from various source localities, were compared to evaluate their influence on EHEC transference from soil to lettuce via the contaminated blade of the harvest knife. Stem-contact cut is used to describe the procedure in which the blade was used to cut only the stem without touching the edible portion of the lettuce head; medium-contact cut refers to the use of the blade to cut off the stem along with a portion of the lettuce head approximately 1 cm above the stem, resulting in contact of the blade with a small portion of the edible lettuce; and heavy-contact cut refers to the use of the blade to cut off the stem along with a portion of the lettuce head approximately 2 cm above the stem, resulting in contact of the blade with a larger portion of the edible lettuce. The portions of the iceberg lettuce head affected by the three cutting scenarios are illustrated in Fig. 2 by coating the blades with dye before making the cuts. The three cutting methods were compared using clay soil with 25% WC, contaminated with 10^4 MPN/g EHEC to inoculate the blade as previously described. Each contaminated blade was used to cut three successive heads of lettuce with one of the three cutting scenarios. During all other tests, including inoculum level, soil type, and soil moisture content, the stem-contact cutting method was used to cut the lettuce heads from their stems.

2.5. Lettuce sampling and E. coli O157:H7 enumeration

Worst case and realistic fresh-cut processing scenarios were simulated by two methods of sampling and recovery of *E. coli* O157:H7 on lettuce. In the worst case scenario, approximately 25-g of tissue was excised from the inside edge of the cored area (about 0.5 cm deep from the original edge) with a sterile knife. Realistic fresh-cut processing conditions were simulated by cutting the entire cored-lettuce heads into 2.5-cm square slices using a lettuce cutter (Silver King Kutlett, Minneapolis, MN). Slices were mixed, and three 25-g samples were randomly taken from each lettuce head. While outcomes using these two sampling methods were compared in the coring ring studies, cutting blade studies used only the worst case scenario sampling method.

Lettuce samples were homogenized in sterile filter stomacher bags with 125 ml TSB supplemented with 200 μg/ml ampicillin using a Stomacher blender (Seward 400 Biomaster, Brinkmann Seward, Ontario, Canada) for 2-min at 230 rpm. An 8-tube modified MPN

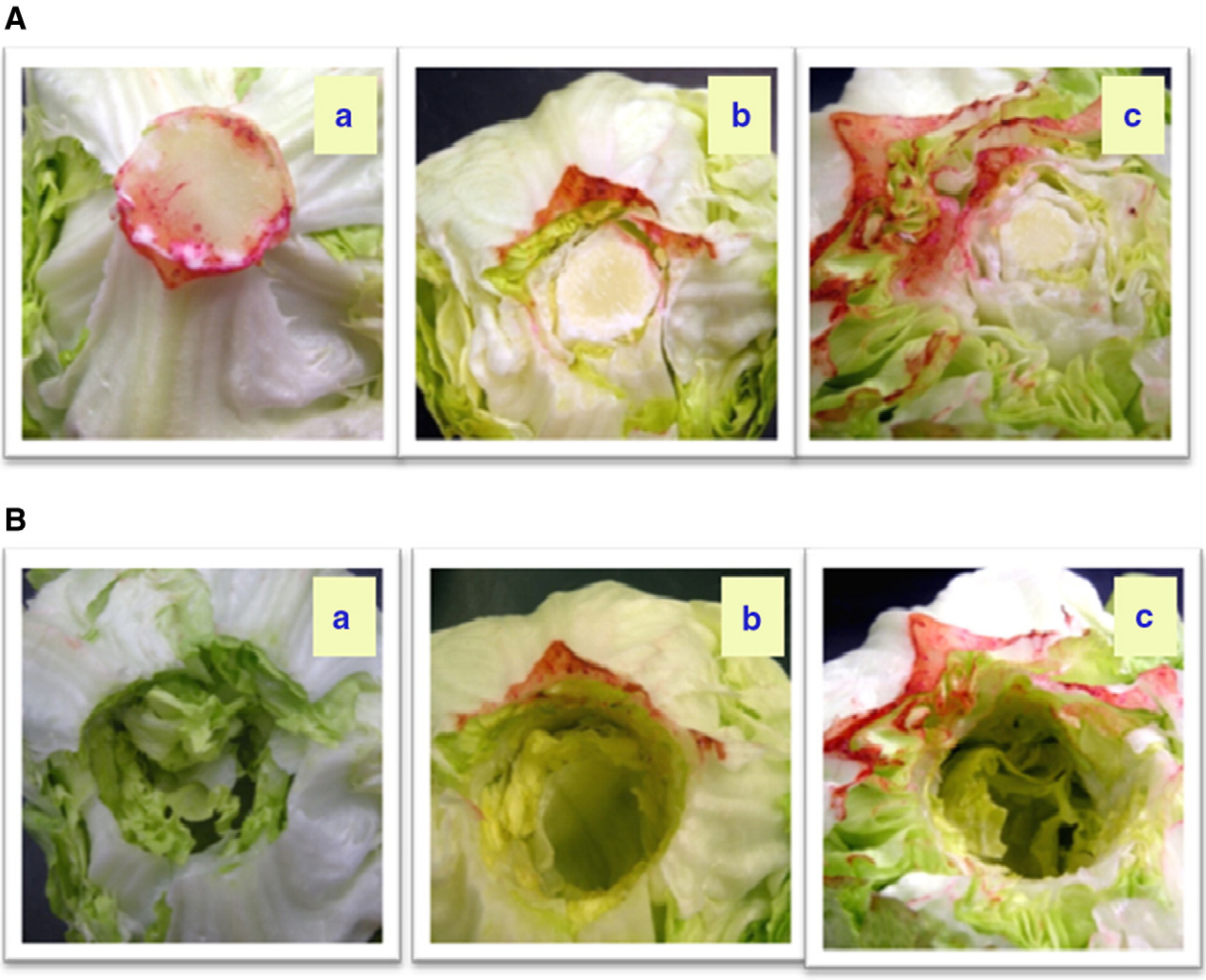

Fig. 2. Demonstration of the effect of cutting blade (stained with a red dye) to lettuce contact on transference of contaminants to the harvested lettuce. a—Stem-contact cut; b—Medium-contact cut; c—Heavy-contact cut. A. Before coring. B. After coring.

method was used to enumerate *E. coli* O157:H7 (Nou and Luo, 2010; Luo et al., 2002). Briefly, eight 3 ml aliquots of the filtered homogenate were added to eight individual microplate wells, followed by serial dilutions with TSB supplemented with 200 μg/ml ampicillin. The microplate was covered with sterile gas permeable film (Breathe-Easy Sealing Membrane, RPIC, Mt. Prospect, Illinois) and incubated overnight at 37 °C. The turbidity of each well after incubation was recorded. Confirmation of *E. coli* O157:H7 growth in turbid wells was accomplished by transferring 3 μl samples from each well to cefixime-tellurite Sorbitol MacConkey (CT-SMAC) agar plates prepared according to manufacturer's directions (Becton Dickenson & Co., Sparks, MD) and supplemented with 100 μg/ml ampicillin. Plates were incubated overnight at 37 °C, and the presence of colonies showing typical green fluorescence was recorded. Serological testing using a rapid RIM™ *E. coli* O157:H7 latex agglutination assay (Remel Inc., Lenexa, KS) was also used periodically for further confirmation of sample *E. coli* O157:H7 colonies. An MPN calculator (VB-6 version) was used to compute the MPN cell count for each sample (Anon, 2010). This method allowed a detection limit of 0.36 MPN/g.

2.6. Experimental design and statistical analysis

Preliminary experiments were done to establish effective inoculation methods and concentrations and to determine the number of successive heads of lettuce contaminated at various inoculum densities. Experiments on the coring ring had four factors: five inoculum levels, three successive heads cored with each knife, two sampling methods, and two inoculation methods.

Experiments on the cutting blade had five factors: five inoculum levels, two soil types with three moisture levels for one soil and five moisture levels for the other soil, three successive heads cored with each knife, and three coring knife contact scenarios. Values reported for each treatment in both experiments are the means of three replicate trials. Analysis of variance was conducted using the general linear model (GLM) of Statistical Analysis Software (SAS Inst. Inc., Cary, NC) and t-tests were used to determine differences among means at $\alpha = 0.05$.

3. Results and discussion

3.1. Coring ring studies

The coring ring of the lettuce coring knife directly contacts the edible portions of the harvested lettuce which are processed for packaging. Therefore, pathogens could be transferred to the lettuce by the coring ring during core removal. Since the contact between coring ring and the lettuce is concentrated at the cored region, pathogen detection on lettuce may vary with the amount of tissue adjacent to the core included in the portion of lettuce sampled. Therefore, the effects of the coring ring on pathogen transference, as well as the impact of two sampling methods on pathogen detection were evaluated.

3.1.1. The effect of contamination level and sampling method on detection of pathogen transfer via coring ring

When the worst case sampling method was employed (Table 1), the pathogen was detectable on 1 out of 3 first lettuce heads at an inoculum level of 10^1–10^2 MPN/ring, on all first lettuce heads, but on only 1 out of 3 second lettuce heads at an inoculum level of 10^2–10^3 MPN/ring and on all first and second lettuce heads but on only 1 out of 3 third lettuce heads at an inoculum level of 10^3–10^4 MPN/ring. However, when an entire lettuce head was sampled, the detection of EHEC required more inoculum on the coring knife than it did for the worst case scenario. EHEC was detectable on 4 of 9 first cored (of three sequential) lettuce heads cut with a ring contaminated with EHEC at 10^3–10^4 MPN/ring, and on 9 of 9 first and 8 of 9 second heads cored with a ring initially contaminated with 10^4–10^5 MPN/ring (Table 2).

Table 1
E. coli O157:H7 transference from coring ring to lettuce as impacted by the inoculation level. 25-g samples taken from the cored region (Worst case).

Target inocula MPN/ring	Rep	Actual inocula MPN/ring	EHEC recovered on lettuce (MPN/g lettuce)		
			1st Head	2nd Head	3rd Head
10^3–10^4	1	5400	160	65	7.9
	2	2976	160	28	0.4
	3	6447	460	9.8	2.1
10^2–10^3	1	520	9.1	3.4	-
	2	298	3.4	-	-
	3	645	14	4.3	0.4
10^1–10^2	1	31	1.6	-	-
	2	32	0.9	-	-
	3	62	1.6	0.4	-
10^0–10^1	1	3.2	0.4	-	-
	2	2.2	-	-	-
	3	6.2	-	-	-

- Refers to undetectable at a detection limit of 0.36 MPN/g lettuce.

3.1.2. The effect of soil contamination level and sampling method on detection of pathogen transfer via coring ring

Coring ring studies using EHEC-inoculated soil contamination yielded results similar to those using direct liquid inoculation. When the worst case sampling method was used (Table 3), transference was detectable on 3 of 9 first lettuce heads cut with a coring ring contaminated with EHEC at 10^1–10^2 MPN/g soil, 8 of 9 first, 2 of 9 second and 1 of 9 third lettuce heads cut with a coring ring contaminated with EHEC at 10^2–10^3 MPN/g soil. In contrast, using the real-life scenario method, EHEC was not detected on any lettuce heads when the coring ring was contaminated with EHEC at 10^2–10^3 MPN/g and only detected on the first of three lettuce heads cut with a coring ring contaminated with EHEC at 10^3–10^4 MPN/g soil, and only on the first and second lettuce heads cut with a coring ring contaminated with EHEC at 10^4–10^5 MPN/g soil (Table 4).

Sampling method plays an important role in the sensitivity of pathogen detection from contaminated iceberg lettuce. In the worst case scenario, samples comprised only lettuce tissues which were in close contact with the coring rings, and where EHEC transferred from the coring ring is mainly concentrated. Therefore, detection sensitivity in this region is high. In the real-life scenario, contaminated lettuce tissues from coring ring-contact areas are mixed with the entire head of lettuce, including tissues that were not contacted directly by the contaminated ring, thus diluting the contamination and reducing the probability of detecting EHEC in a single sample. However,

Table 2
E. coli O157:H7 transference from coring knife ring to lettuce as impacted by the inoculation level. 3 sets of 25-g random samples from whole lettuce head (Real-life scenario).

Target inocula MPN/ring	Rep	Actual inocula MPN/ring	EHEC recovered on lettuce (MPN/g lettuce)								
			1st Head			2nd Head			3rd Head		
			Sample set			Sample set			Sample set		
			1	2	3	1	2	3	1	2	3
10^3–10^4	1	3600	250	2.8	1.6	1.4	1.4	2.1	-	-	-
	2	2400	1.6	260	2.1	1.9	0.4	-	-	-	-
	3	6200	460	3.4	2.1	2.1	2.1	0.9	-	-	-
10^2–10^3	1	320	0.4	-	-	-	-	-	-	-	-
	2	220	-	0.4	-	-	-	-	-	-	-
	3	520	0.4	0.4	-	-	-	-	-	-	-
10^1–10^2	1	15	-	-	-	-	-	-	-	-	-
	2	32	-	-	-	-	-	-	-	-	-
	3	22	-	-	-	-	-	-	-	-	-

- Refers to undetectable at a detection limit of 0.36 MPN /g lettuce.

Table 3
Coring ring-assisted *E. coli* O157:H7 transference to lettuce as impacted by soil contamination level. 25-g samples from cored region (worst case scenario).

Target inocula (MPN/g)	Rep	Actual inocula (MPN/g)	*E. coli* O157:H7 recovered on lettuce (MPN/g lettuce)								
			1st Head			2nd Head			3rd Head		
			Sample set			Sample set			Sample set		
			1	2	3	1	2	3	1	2	3
10^4–10^5	1	19500	25	160	98	2.1	16	25	0.4	6.5	6.5
	2	22000	140	98	91	19	9.8	6.5	4.6	9.8	2.5
	3	18000	110	70	91	9.9	3.4	4.6	1.6	3.4	0.9
10^3–10^4	1	8300	9.8	6.5	4.6	1.6	1.6	0.4	-	0.4	-
	2	8700	6.5	6.5	4.3	1.6	1.6	0.4	0.4	0.4	-
	3	7700	4.6	5.4	4.3	0.9	1.6	0.4	-	0.4	-
10^2–10^3	1	325	2.5	1.4	0.9	0.9	-	-	0.4	-	-
	2	310	1.6	0.9	0.9	0.4	-	-	-	-	-
	3	210	-	0.4	0.9	-	-	-	-	-	-
10^1–10^2	1	31	0.4	-	0.4	-	-	-	-	-	-
	2	31	-	-	0.4	-	-	-	-	-	-
	3	23	-	-	-	-	-	-	-	-	-
10^0–10^1	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-

- Refers to undetectable at a detection limit of 0.36 MPN/g lettuce.

mixing contaminated with uncontaminated lettuce also spreads contaminants, and thereby increases the number of potentially contaminated packages of product.

3.2. Cutting blade studies

Since lettuce heads grow close to the soil and the blade is used to cut the lettuce head from the stem, the proximity of the blade to soil during harvest, and the adherence of soil to lower portions of the plant, result in a greater potential for contact with the cutting blade than with the coring ring. Thus, the cutting blade represents a critical vehicle for pathogen transfer. However, its potential to transfer pathogens to the edible portions of the lettuce is relatively less than that of the ring, because it has minimal contact with the remaining lettuce tissues, when used properly. Factors that may potentially affect pathogen transference from soil to the edible portion of lettuce may include soil pathogen contamination levels, soil type, soil moisture content, and variations in commercial CIF harvesting methods. The effects of these factors are thus evaluated in this series of studies.

3.2.1. Pathogen transfer via cutting blade as impacted by soil type and water content

The effects of soil type and soil water content on pathogen transfer were evaluated by inoculating the cutting blades with soil contaminated

Table 4
Coring ring-assisted *E. coli* O157:H7 transference to lettuce as impacted by soil contamination level. 3 sets of 25-g random samples from lettuce (Real-life scenario).

Target inocula MPN/g soil	Rep	Actual inocula MPN/g soil	*E.coli* O157:H7 recovered on lettuce (MPN/g)								
			1st Head			2nd Head			3rd Head		
			Sample set			Sample set			Sample set		
			1	2	3	1	2	3	1	2	3
10^4–10^5	1	28000	9.1	16	260	3.4	4.3	1.6	-	-	-
	2	22000	9.1	6.5	99	2.1	1.6	1.6	-	-	-
	3	25000	4.6	9.1	140	0.89	1.6	2.7	-	-	-
10^3–10^4	1	4200	0.89	0.8	0.4	-	-	-	-	-	-
	2	3800	0.4	0.89	0.4	-	-	-	-	-	-
	3	3600	-	0.89	0.8	-	-	-	-	-	-
10^2–10^3	1	380	-	-	-	-	-	-	-	-	-
	2	380	-	-	-	-	-	-	-	-	-
	3	240	-	-	-	-	-	-	-	-	-

- Refers to undetectable at a detection limit of 0.36 MPN /g lettuce.

Table 5
Influence of water content of clay soil on EHEC transference.

Water content (%)	Attached soil (g/blade)	Rep	Estimated inocula MPN/g soil	EHEC recovered on lettuce (MPN/g lettuce)		
				1st Head	2nd Head	3rd Head
30	31.21	1	260000	2600	16	2.5
	31.26	2		4300	46	9.1
	30.58	3		2800	9.8	1.6
25	22.68	1	360000	210	1.6	-
	21.23	2		140	0.89	-
	22.14	3		280	2.7	-
20	10.12	1	180000	-	-	-
	9.87	2		-	-	-
	10.66	3		-	-	-
15	0.81	1	230000	-	-	-
	0.99	2		-	-	-
	1.02	3		-	-	-
10	0.06	1	120000	-	-	-
	0.05	2		-	-	-
	0.05	3		-	-	-

- Refers to undetectable; below detection limit of 0.36 MPN /g lettuce.

at 10^5 MPN EHEC/g with WC of 5–15% for sandy soil or 10–30% for clay soil. As shown in 5, raising the soil WC increased the amount of soil contaminating the blades and resulted in greater transference of EHEC from soil-to-blade and from blade-to-lettuce head. EHEC were detectable on all three lettuce heads or the first and second lettuce heads cut with a blade contaminated with clay soil having 30% or 25% WC, respectively. Although we tried carefully to avoid contacting the edible portion of lettuce with the soiled blade, when the clay soil WC was 25% or greater, it was extremely difficult to avoid contact between the soiled-knife blade and the edible lettuce tissue. These high WC soils represent the environmental condition when lettuce is harvested soon after rain or application of irrigation water (especially for clay soil). Therefore, extreme caution must be used during lettuce harvesting to avoid knife contact with soil to reduce the potential for pathogen transference, especially when the field soil is recently wetted. Furthermore, clay soils have been shown to increase the persistence and activity of *E. coli* O157:H7 (Gagliardi and Karns, 2002). In contrast, pathogen transference from soil to cutting blade and then to lettuce differed significantly when sandy soil was used (data not shown). EHEC was not detected on lettuce heads when the knife blade was contaminated with sandy soil, even at 15% WC (very wet for sandy soil).

3.2.2. Pathogen transfer via cutting blade as impacted by cutting-knife contact scenario

The risk of pathogen transfer to lettuce is highly dependent on the extent and location of contact between harvesting blade and lettuce tissues. As shown in Table 6 and Fig. 2, EHEC transference from soil to lettuce was influenced significantly by different cutting-knife contact intensities. When the cutting blade was contaminated with soil

Table 6
Influence of cutting method on EHEC transference in clay soil.

Cut method	Rep	EHEC recovered on lettuce (MPN/g lettuce)		
		1st Head	2nd Head	3rd Head
Stem-contact	1	-	-	-
	2	-	-	-
	3	-	-	-
Medium-contact	1	2.3	0.68	-
	2	1.7	0.4	-
	3	3.3	0.78	-
Heavy-contact	1	460	98	2.5
	2	660	110	34
	3	250	65	4.6

- Refers to undetectable; below detection limit of 0.36 MPN /g lettuce.
Estimated inoculum cell density: 52667 MPN/g soil; water content: 25%.

containing 10^4 MPN EHEC/g and knife contact was limited to the lettuce stem, no positive samples were detected on any of the three heads successively cut with the blade. However, EHEC transference was detectable on the first and second cut lettuce heads when medium-contact occurred between knife and lettuce heads and on all three heads when cut with heavy-contact at this same inoculum level. Different cutting contact scenarios result in different amounts of the edible portion of iceberg lettuce heads being impacted by the knife. The larger the contact area, the more soil that potentially can adhere to the edible part of lettuce, and the more EHEC is transferred. The risk of pathogen transfer from blade to the edible portion of lettuce increases significantly as the contact between the blade and the edible portion of lettuce increases.

The study by Taormina et al. (2009) used a sampling method similar to the worst case scenario used here, with only a slightly larger sampled region, resulting in their reporting of a higher risk of contamination than we saw in our real life scenario. In their study, only the blade was contaminated, yet their result showed consistently strong transfer of E. coli cells all the way out to 10 sequentially cored heads. In our own studies we found that the transfer of E. coli cells tapered off significantly with each sequentially cored head. McEvoy et al.(2009) also cored 10 successive heads with a contaminated coring knife, but had higher starting inocula levels and did report not only significant reduction of cell counts with number of heads cored, but also a reduction in number of positive samples found after the sixth head cored with the same contaminated knife.

4. Conclusions

The current California Leafy Green Marketing Agreement (LGMA, 2009) calls for attention to CIF harvesting to minimize pathogen transfer; yet, detailed information is needed as to how to minimize pathogen contamination from soil during harvesting/coring operations. This study comprehensively evaluated the potential risk of pathogen transference from soil to harvested lettuce via a contaminated harvesting knife coring ring and cutting blade by simulating microbial contamination and knife contact points as they may occur during lettuce CIF harvesting practice. The cutting blade and coring ring play significantly different roles in pathogen contamination and transference from soil to lettuce. Under the current CIF harvesting practice, the cutting blade has higher potential to be contaminated by the soil, but less opportunity to transfer pathogens to harvested lettuce. On the contrary, the coring ring has less potential to be contaminated by soil; however, once contaminated, it has much higher potential to transfer pathogens to the harvested lettuce. Since the cutting blade is used to cut lettuce from the stem that touches the ground, it is important to minimize the potential for the cutting blade to contact soil whenever possible. However, blade contact with soil may be inevitable at some point during harvesting under the current harvesting practice. Minimizing contact between the blade and edible portions of the lettuce plays a vital role in minimizing pathogen transfer.

Acknowledgments

We wish to thank Eric Brennan from USDA-ARS (Salinas, CA) and Sam Liu from Taylor Farms (Salinas, CA) for providing the soil samples, Dr. Xiangwu Nou for critical reading of the manuscript. This project was sponsored by California Leafy Green Research Board and Center for Produce Safety (CPS project # 2009-48).

References

Anon, 1996. Spotlight on trimmed & cored lettuce. Fresh Cut Magazine 4, 17.
Anon, 2001. Field-cored Lettuce-Best Practices. Available at http://www.unitedfresh.org/assets/files/GR/Field_Cored_Lettuce_Best_Practices.pdf accessed April 12, 2010.
Anon, 2010. http://www.i2workout.com/mcuriale/mpn/index.html.
Barker-Reid, F., Harapas, D., Engleitner, S., Kreidl, S., Holmes, R., Faggian, R., 2009. Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. Journal of Food Protection 72, 458–464.
Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology 12, 2385–2397.
Brown, R.S., Rizzo, E.D., 1999. Apparatus and methods for washing the cored areas of lettuce heads during harvest, United States Patent #6,298,865 B1
Brown, R.S., Rizzo, E.D., 2001. Methods for washing cores of cored lettuce heads. United States Patent #6,196,237.
CDHS (California Department of Health Services), 2007. Investigation of an *E. coli* O157:H7 outbreak associated with consumption of Dole brand pre-packaged baby spinach manufactured by Natural Selection Foods: September 2006–March 2007.
Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J., Mandrell, R.E., 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS One 2 (11), e1159. doi:10.1371/journal.pone.0001159.
De Groot, P., Tracy, J., Willoughby, F., 2008. Systems and methods for automatically picking and coring lettuce and cabbage. United States Patent #7,395,653.
Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007 — the problems with fresh produce: an overview. Journal of Applied Microbiology 105, 317–330.
FAO/WHO, 2008. Microbiological hazards in fresh leafy vegetables and herbs. Expert Panel Report. Microbiological Risk Assessment Series, 14. WHO Press (138 pp.).
Fratamico, P.M., Deng, M.Y., Strobaugh, I.P., Palumbo, S.A., 1997. Construction and characterization of *Escherichia coli* 0157:H7 strains expressing firefly luciferase and green fluorescent protein and their use in survival studies. Journal of Food Protection 60, 1167–1173.
Gagliardi, J.V., Karns, K.S., 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. Environmental Microbiology 4, 89–96.
LGMA (Leafy Green Market Agreement), 2009. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. Available at http://www.caleafygreens.ca.gov/members/documents/LGMAAcceptedFoodSafetyPractices01.29.10.pdf accessed on March 29, 2010.
Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E.R., Feng, H., Abadias, M., Conway, W.S., 2002. Determination of free chlorine levels needed to prevent cherichia coli O157:H7 cross-contamination during fresh-cut produce wash. Journal of Food Protection 74, 352–358.
Lynch, M.F., Tauxe, R.V., Hedberg, C.W., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology and Infection 137, 307–315.
McEvoy, J.L., Luo, Y., Zhou, B., Feng, H., Conway, W.S., 2009. Potential of *Escherichia coli* O157:H7 to grow on field-cored lettuce as impacted by postharvest storage time and temperature. International Journal of Food Microbiology 128, 506–509.
Nou, X., Luo, Y., 2010. Washing whole leaves prior to cutting improves microbial reduction efficacy and prevents pathogen cross-contamination during fresh-cut lettuce processing. Journal of Food Science 75, M283–M290.
Suslow, T.V., Oria, M.P., Beuchat, L.R., Garrett, E.H., Parish, M.E., Harris, L.J., Farber, J.N., Busta, F.F., 2003. Production practices as risk factors in microbial food safety of fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety 2, 38–77.
Takeuchi, K., Matute, C.M., Hassan, A.N., Frank, J.F., 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. Journal of Food Protection 63, 1433–1437.
Taormina, P.J., Beuchat, L.R., Erickson, M.C., Ma, L., Zhang, G., Doyle, M.P., 2009. Transfer of *Escherichia Coli* O157:H7 to iceberg lettuce via simulated field coring. Journal of Food Protection 72, 465–472.