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CPS - CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

FINAL PROJECT REPORT, DUE APRIL 30, 2010

Project Title
Minimizing pathogen transference during lettuce harvesting by optimizing the design of the harvesting device and operation practices

Project Period
April 1, 2009 through April 30, 2010

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Objectives
Objective 1: Determine the pathogen levels required for pathogen transference to the edible portions of lettuce via contaminated coring knives.

Objective 2: Reduce the risk of coring knife pathogen transference by developing improved coring knife design and sanitation procedures.

Objective 3: Eliminate the potential for coring knife contamination via soil contact by separating the cutting and coring process.

Objective 4: Identify post-harvest handling practices that can be used to effectively manage the potential food safety risks during CIF.

1. Technical Report

1.1. Executive Summary

This one year project comprehensively evaluated the potential risk of pathogen transference from contaminated soil to lettuce head via a harvesting knife during lettuce coring-in-field (CIF) harvesting, and potential approaches for risk reduction. The project team identified the following three major factors that are essential for pathogen transfer from contaminated soil to lettuce via a coring knife: A) sufficiently contaminated soil; B) contact between a coring knife and the contaminated soil; and C) contact between contaminated coring knife and the edible portions of the lettuce. Factor A was addressed by examining the *E. coli* O157:H7 (EHEC) transference as impacted by soil contamination levels, as well as soil type and moisture conditions. *E. coli* O157:H7 inoculation levels covered a broad range (1-100,000 MPN/g soil) so that the data obtained can be used to perform risk assessment under realistically low soil contamination levels, and to compare to results in the literature that often use excessively high inoculation levels. For factors B and C, we investigated the roles of different portions of a harvesting knife, i.e. coring ring vs. cutting blade, and their design on their potential for being contaminated by soil, and for transferring the contaminants to the edible portions of lettuce during harvesting. The principle investigator of the project team visited CIF harvesting operations in California, and the team evaluated lettuce harvested from California, Florida, and Mexico in order to assess the effects of variations in coring method on transference and to develop practical recommendations for risk reduction. We observed that the harvesting knife design currently used in the industry has a rough weld joint that facilitates pathogen adherence and attachment and makes the knives difficult to disinfect. Two prototypes of harvesting knives with improved food safety features were developed and field tested. Very positive feedback was received from the industry. Additionally, the project team tested various technologies to enhance coring knife disinfection.

1.2. Major Accomplishments by USDA Team

This project was completed collaboratively by two project teams: USDA-ARS (responsible for objective 1, 3, and 4) and UIUC (responsible for objective 2). The work originally proposed in objective 3 to assess the feasibility of reducing pathogen contamination and transference by separating cutting from coring through automation was not carried out as it was not funded.

1.2.1. Research Activities and Testing Methodologies:

The potential for pathogen transference from soil to the edible portions of lettuce via a contaminated lettuce coring-harvesting knife was thoroughly investigated. Factors considered included soil pathogen contamination levels (1-100,000 MPN/g soil), soil type and moisture level, the variations in commercial CIF harvesting methods, the blade vs. ring of the coring knife, and the portions of lettuce to be consumed. The pathogen quantification method used was the Most Probable Number (MPN) enrichment method, modified to multi-well deep micro plate format (8-tube 5-dilution) to provide a low detection threshold of 0.36 MPN/g soil or lettuce. This method was validated in a preliminary study; and comparative counts were obtained from both plating (CFU/g) and MPN (MPN/g) methods. An attenuated *E. coli* O157:H7 strain (CDC B6914; with double markers: pGFP and Ampicillin resistance) was used throughout the studies.

1.2.2. Key Findings:

1) **Contamination pathways and critical factors**: The currently widely used lettuce coring knife consists of two major components, i.e. cutting blade (Fig. 1A) and coring ring (Fig. 1B). The cutting blade is primarily used to cut the lettuce heads from the stems (Fig 2A) while the ring is used to remove the cores (Fig. 2B). This generates a clean and ready-to-process lettuce head with cores removed (Fig. 2C). The blade is used close to the soil and therefore has a high likelihood of becoming contaminated. The ring is used following the blade and therefore may become contaminated by contacting lettuce already contaminated. However, if the blade is used only on the lettuce stem and not on the edible portion of lettuce, the ring will be able to remove the core without contacting or spreading the contamination from the stem. On the other hand, if the blade cuts through and leaves soil on the edible portions of lettuce, then the ring will drag the contaminants into the head of lettuce along the core path. These suggest that possible pathways of contaminant transfer from harvesting tool to lettuce may include: 1) from knife blade to lettuce when lettuce head is cut from the plant stem, 2) from knife blade contact with lettuce tissue and transfer along the cored path, and 3) from the coring ring directly to core path of lettuce tissue. Major factors impacting pathogen transference from contaminated soil to lettuce via a harvesting knife were identified as A) sufficiently contaminated soil; B) contact between a coring knife (blade or ring) and the contaminated soil; and C) contact between contaminated coring knife and harvested lettuce. These factors are further impacted by soil type, soil moisture level, CIF harvesting method, and knife design. Figure 3 demonstrates the path of lettuce contamination via a coring ring (stained in blue) and cutting blade (stained in red).

2) **Impact of soil contamination level on pathogen transfer via coring ring:** Effect of soil contamination level on pathogen transfer via coring ring was evaluated by dip-inoculating coring ring of the knife (Agricultural Supplies Inc., Salinas, CA, USA) in 10% moist EHEC-spiked Salinas sandy-loam soil. Lettuce samples (25g) were taken from tissue in the cored region (method A; worst case scenario) or three sets of 25g random samples from the entire lettuce head after slicing (method B; real-life fresh-cut processing scenario). Test results show that low concentrations (0.4 MPN/g) of EHEC were detected on some of the 1st of 3 heads in a series cut with a knife contaminated with EHEC ≥10-100 MPN/g soil (Table 1), for method A. With equivalent inoculation, EHEC detection frequency was much lower in the real life scenario than in the worst case scenario. With the real life scenario, EHEC was found on only the 1st lettuce head cut with a knife contaminated with EHEC ≥1,000-10,000 MPN/g, or the 1st and 2nd of 3 lettuce-heads cored with a knife contaminated with EEHC ≥10,000 -100,000 MPN/g soil (Table 2). No EHEC was detected on remaining cored-lettuce samples.

3) **Impact of soil contamination level and blade to lettuce contact on pathogen transfer via the cutting blade:** In addition to soil EHEC contamination level, the risk of pathogen transfer to lettuce is highly dependent on the extent and location of contact between harvesting blade and lettuce tissues. Since lettuce heads grow close to the soil and the blade is used to cut the lettuce head off 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 higher potential for the cutting blade to contact soil than the coring ring. However, the blade's potential to transfer pathogens to the edible portions of the

lettuce is much smaller than that of the ring (comparing Tables 1 and 3) because its contact with the lettuce tissues that remain on the harvested lettuce head is minimal, when used properly (as shown in Fig. 4A). However, 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 (Table 4, Fig. 4B, and 4C). Figure 5 further demonstrates the impact of blade to lettuce contact on the transference of contaminates to the harvested lettuce.

4) **Soil type and moisture level on pathogen transference:** Three types of soils from lettuce growing field were tested. These include sandy-loam and loamy-clay soils obtained from typical lettuce growing fields in various regions of Salinas, CA and Yuma, AR. Moisture tested ranged from 10, 15, 20, 25, and 30%, covering soil conditions from very dry to very wet. As shown in Table 5, the amount of soil contaminating the blades increased with the soil moisture level. Although the researchers tried carefully not to contaminant the lettuce with the blade, when the soil levels reached 25% or higher, it was extremely difficult to avoid contaminating the lettuce. This may represent a situation that occurs when the lettuce is harvested right after rain or after application of irrigation water. Therefore, extreme caution must be taken during lettuce harvesting so as not to allow the knife to contact soil in order to avoid pathogen transference, especially when the field soil is wet due to rain or irrigation.

5) **Current commercial harvesting practice evaluation:** During a visit to Taylor Farm CIF harvesting practice (September 2009), a project team member observed that the harvesters cut the lettuce from the plant stem using the blade portion of the harvest knife (Fig 1A), and then removed the cores using the circular (ring) portion of the knife (Fig 1B). This resulted in a cleanly cut/cored lettuce with minimum contact between blade and edible portions of the lettuce (Fig 1C). Similar practice was observed during a prior visit to CIF harvesting at River Ranch Fresh Foods. Please note, during the visit, harvest crews were closely watched by their supervisors, and by the visitors; and the soil condition was reasonably dry; Iceberg lettuce was suitably mature for harvest.

   To further evaluate CIF harvesting practices under normal (un-audited) conditions, samples from three harvesting crews/regions were evaluated. All samples harvested in California (n =15; Fig 6A) had appearance similar to samples observed during the prior field visit. This suggests that the CIF harvest operations in California follow the same practices as observed. However, samples received from Mexico (n= 21) and Florida (n=26) suggest that harvest crews' practice may be different in these regions. A large percentage of samples from Mexico appeared to have the cores cut out with the blade (Fig 6B) that was also used to cut the lettuce from the stem, thereby increasing the potential for soil to contact the edible portions of the lettuce. Samples from Florida also appeared to have some blade to edible lettuce contact, although better than those from Mexico; however, numerous samples had soil present on both the leaves and the cut/cored areas (Fig 6C). Further tests also showed that samples from Florida had the highest total aerobic plate count (APC), while the samples from California had the lowest APC (near 3 log CFU/g difference) even though samples from California were evaluated 3 days post harvesting while the samples from Florida were evaluated only 1 day post harvesting. The weather record indicated that the harvesting region in Florida had received rain for 13 hours (a total of 0.66 inch) the day prior to the lettuce harvest. While we are not sure if other factors beyond the rain also contributed to the presence of soil on harvested lettuce from Florida and its abnormally large bacterial counts, clearly cutting knives are much more prone to soil contact when CIF harvest is carried out in wet soil than in dry soil (Table 5).

1.3. Major Accomplishments by UIUC Team

Technologies to enhance coring knife disinfection that could be used in the field were tested; new prototypes of coring knives with improved food safety features were developed and field-tested.

1.3.1. Major Activities and Testing Methodologies:

The UIUC project team examined in detail the design features of current commercially used coring knives in relation to soil and pathogen adherence and attachment. Given the fact that harvest crews may use the coring knives for about 2 hr before disinfecting them during break, the coring knives may have a significant amount of soil and lettuce extract accumulated on them during 2 hr. This condition was thus simulated by the preparation of *E. coli* O157:H7 inocula mixed with lettuce extract and soil slurry. The coring rings were dip-inoculated in this mixture containing 10^6 CFU/ml EHEC cells for 1 minute followed by air-drying at 22°C for 2.0 hours. Wetted sterile cotton swabs were then used to wipe different portions of the knife surface, followed by stomaching in sterile PBS solution, and enumerating with a direct plating method. The detection limit for the microbial analysis was 12.5 CFU/cm^2 for the swabbed surface areas. To test the effect of chlorine in combination with ultrasound or surfactants on improving disinfection efficacy, inoculated knives were submerged in different concentrations of chlorine solution (1, 10, 50, 100, and 200 ppm free chlorine), with or without ultrasonication (25 kHz, 500W/L) for 0.5, 1, or 2 min, or a surfactant (Tween80) at concentrations of 10, 100, and 1,000 ppm.

Two prototype coring knives with improved food safety features were developed. The first prototype was made using a no-joint design to connect the cutting blade and the coring ring. The cutting blade was the same as the current commercial coring knife; and the coring ring (with the tang) was made from a 316 stainless steel tube using wire EDM (Electrical Discharge Machining). The coring knife handle was made of Vero materials, and was created using a Rapid Prototyping technique. The cutting blade and coring ring were assembled together through the tangs by tungsten inert gas (TIG) welding, and inserted in the handle. The two sides of the handle were adhered by J-B weld epoxy, and clamped together for 24 hours. The drawings of the parts and the assembly are included in Appendix 5. The second prototype was essentially the same as the commercial coring knife except that the welded joint was mechanically polished.

1.3.3. Key Findings:

1) **Improving coring knife disinfection by combining ultrasound with chlorine:** Examination of the design of the current commercially used coring knife in relation to EHEC adherence and attachment revealed that the rough surface connecting the coring ring and the shaft harbors significantly more EHEC than the smooth surfaces (Fig. 7) and has much less disinfection efficacy. When coring knives were washed in chlorine alone, at least 50 ppm free residual chlorine was needed in the wash solution to inactivate EHEC on welded knife parts, whereas 10 ppm residual chlorine was needed for the smooth areas (Fig. 8). However, combining ultrasound with chlorine significantly improved chlorine efficacy on pathogen inactivation in the rough

surfaces. When ultrasound for 1 min was combined with chlorine, pathogen inactivation to undetectable levels was achieved at 1 ppm residual free chlorine on all knife parts. Adding surfactant (Tween 80) to the chlorine solution did not improve the inactivation of EHEC cells on the welding joint of the knife (Fig. 9).

2) **Prototyping new coring knives with improved food safety features**: To further address disinfection issues associated with rough welds (Fig. 10A), two prototype coring knives were developed and tested. The prototypes included a no-joint design (Fig. 10B) and a polished weld joint design (Fig. 10C). Polishing weld joints may be an option for retrofitting the currently used knives in the industry. Test results show that the two prototype knives harbored significantly fewer EHEC cells on the coring rings than the commercial ones (Fig. 12A) and facilitated more effective EEHC inactivation by chlorine (Fig. 12B). Prototype 1 (Fig. 11) was field tested during Taylor Farm's CIF harvest. Positive feedback was received from the harvest crew leader.

1.4. Project Summary and Recommendations:

1) Pathways and critical factors in relation to pathogen transference from soil to lettuce were identified. Pathogens transferred from soil to harvested lettuce are a function of pathogen concentration in soil, and the amount of soil present on the harvested lettuce, which are then impacted by a number of factors including lettuce growing and harvesting conditions, CIF lettuce harvesting methods, and knife design and disinfection. Optimizing harvesting practice, and improving harvest knife design and disinfection can significantly minimize this potential risk.

2) The cutting blade and coring ring of a CIF harvest knife play significantly different roles in pathogen contamination and transference from soil to lettuce. Analysis of the current **C**IF harvesting practice observed in California reveals that the cutting blade has higher potential to be contaminated by the soil, but less opportunity to transfer pathogens onto to harvested lettuce. On the contrary, the coring ring has less potential to be contaminated by soil; but much higher potential to transfer pathogens onto the harvested lettuce.

3) The current CA Leafy Green Marketing Agreement 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. Since the cutting blade is used to cut lettuce off the stem that touches the ground, it is important to minimize the potential for the cutting blade to contact soil whenever possible. However, since the blade to soil contact may be inevitable at some point during harvesting under the current CIF practice, avoiding contact between the blade and the edible portions of the lettuce plays a vital role in minimizing pathogen transfer. Field observations of the current California CIF harvesting practices, and random samples received from California indicate that most harvesters in California (at least those harvesting for leading fresh-cut processors) are being careful to minimize contact between blade and soil, and between blade and harvested lettuce. However, evaluation of random samples received from Florida and Mexico suggests that CIF harvesting in these regions need more improvement. A cutting blade that touches soils should NOT be used to touch the edible portions of the lettuce.

4) Harvesting CIF lettuce in a wet field (due to rain, irrigation etc.) represents a great challenge to minimize transfer of soil to harvested lettuce. Extreme care must be exercised to avoid harvest knife contact between soil, and lettuce, if CIF lettuce has to be harvested under this condition.

5) The harvest knives currently widely used in the industry need improvement. The rough weld between the cylindrical ring and its shaft harbors pathogens and is difficult to disinfect. Both of our newly designed knife prototypes harbored fewer pathogens and were easier to disinfect.
6) The ability of ultrasound and surfactant to enhance chlorine disinfection efficacy was tested. While surfactant did not show any improvement on pathogen reduction, ultrasound improved chlorine efficacy for pathogen reduction on the roughly welded surface.

2. What unexpected outcomes resulted from the project, and how did you manage them

USDA Team: Our evaluation of CIF lettuce from three regions was a major turning point for our research. Our submitted proposal was only to evaluate EEHC transfer via the coring ring. However, after learning that the cutting blade had indeed been used to remove the cores in the non-Western US regions, thus creating a major contact point between soil and the edible portion of the lettuce, via the blade, we have expanded our project to include the evaluation of the role of the cutting blade on pathogen transfer. Our studies were also expanded to include the evaluation of soil types and moisture levels on pathogen transfer after we noticed their important roles.

UIUC Team: We noticed that ultrasonication increased the degradation rate of chlorine, probably attributable to the break down and removal of soil particles from the surface of the coring knife. This issue was responded to by increasing the monitoring of the chlorine levels 'before' and 'after' treatment. Also, when we prepared the proposal, we were concerned that the accumulation of soil particles on the tank wall may block the radiation surface of transducer and thus reduce ultrasound efficacy. We were pleased to see that although the accumulation of soil in the ultrasound tank did occur, it did not affect the disinfection efficacy of ultrasound.

3. Collaborative efforts involved in planning and/or implementing this project.

Staff members (Greg McLucas, Sam Liu) at Taylor Farms were especially helpful in providing CIF lettuce harvested from three regions, and soil samples from Salinas, CA. Eric Brennan (USDA-ARS, Salinas, CA) and Jorge Fonseca (Arizona State University, Yuma, AR) also helped with providing soil samples. Technicians in the MechSE Machine Shop at UIUC assisted the design and fabrication of the prototypes of lettuce coring knives.

4. Did you have the necessary funds to fully implement this project?

The funds spent on this project exceeded the funds requested. However, since the activities proposed in this project fit the mission of our USDA-ARS in-house research project, we were able to supplement this research with the in-house funds and resources. We do consider that it would have been extremely helpful if we had more travel funds so that more of our project team members could have visited the CIF harvesting practices and conducted more field-related research studies.

5. Describe any changes that occurred to the original budget.

The proposal requested a total budget of $119,125. The project was funded at $103,125. This reflects a budget cut of $16,000 due to the fact that the proposed objective 3 - seperating lettuce cutting from coring via automation was not funded.

6. Give a brief narrative breakdown of how the grant funds were spent.

USDA Team ($76,125)

- Salary for a Visiting Scientist/Postdoctoral Research Associate and a Summer Student Intern.
- Travel for PI Luo and Co-PI Millner to attend CPS symposium (Davis, CA)
- Routine lab wares and consumables (lettuce, multi-channel pipette, tips, media, micro-plates, culture broth, etc.)

UIUC Team ($27,000)

- Salary for a Ph.D. student
- Development and fabrication of the prototypes of lettuce harvesting knives
- Routine lab wares, microbial testing media, and lettuce etc.

7. List publications and presentations resulting from this grant

Appendix 1: An abstract entitled "Ultrasound as an intervention technology for the sanitation of coring knife" was submitted to the IFT and will be presented as poster during IFT's annual meeting (Chicago, IL; 7/2010). No other publications at this stage.
Appendix 2: A PowerPoint presentation file prepared for the annual meeting of CA Leafy Green Research Board (Salinas, CA; 3/2010)
Appendix 3: A draft manuscript titled "Minimizing *Escherichia coli* O157:H7 transference from soil to Iceberg lettuce via a contaminated coring knife".
Appendix 4: A draft manuscript titled "Reducing *Escherichia coli* O157:H7 transference during lettuce harvesting by improving harvesting knife design and disinfection".
Appendix 5: Drawings of the two prototypes of the improved coring knives.

8. Suggestions to CPS

It has been a great pleasure working with CPS. We found that the staff members at CPS are very helpful, and responsive. The RFP that we responded for had a maximum of $125,000 per grant and one year duration. We are glad to see that the recent RFPs from CPS have increased the funding level to $250,000 with two year duration, which we believe are great news to the produce safety research community. So, keep up the good jobs CPS.

Tables and Figures

	
Figure 1. A typical lettuce CIF harvest knife. A. Cutting Blade; B. Coring Ring	Figure 2. Demonstration of a typical lettuce CIF harvesting process

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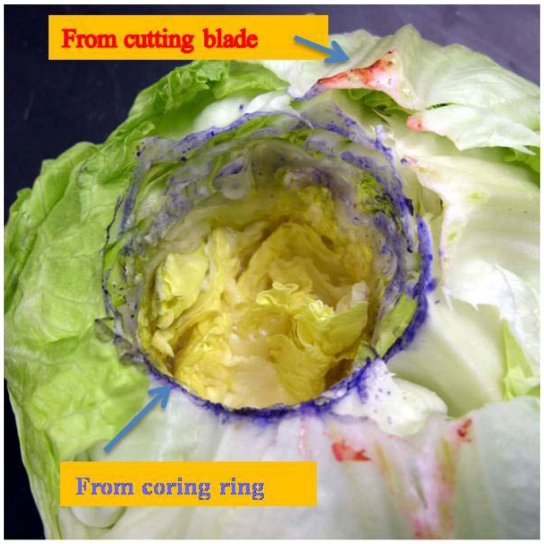

Figure 3. Demonstration of the pathways of contaminants transfer via cutting blade and coring ring.

To distinguish the different roles of cutting blade and coring ring, the blade was stained with red dye, while the ring was stained with blue dye.

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Table 1. 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)	**Trial**	**Actual inocula (MPN/g)**	***E.coli* O157:H7 Recovered on Lettuce (MPN /g lettuce)**								
			1st Head			**2nd Head**			**3rd Head**		
			Replication			Replication			Replication		
			1	2	3	1	2	3	1	2	3
10^4-10^5	1	19,500	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	8,300	9.8	6.5	4.6	1.6	1.6	0.4	ND	0.4	ND
	2	8,700	6.5	6.5	4.3	1.6	1.6	0.4	0.4	0.4	ND
	3	7700	4.6	5.4	4.3	0.9	1.6	0.4	ND	0.4	ND
10^2-10^3	1	325	2.5	1.4	0.9	0.9	ND	ND	0.4	ND	ND
	2	310	1.6	0.9	0.9	0.4	ND	ND	ND	ND	ND
	3	210	ND	0.4	0.9	ND	ND	ND	ND	ND	ND
10^1-10^2	1	31	0.4	ND	0.4	ND	ND	ND	ND	ND	ND
	2	31	ND	ND	0.4	ND	ND	ND	ND	ND	ND
	3	23	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^0-10^1	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND[£]- Not detectable at a detection limit of 0.36 MPN/g lettuce.
Soil: Salinas Sandy-loam; 10% moisture

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Table 2. Coring Ring-assisted *E. coli* O157:H7 Transference to Lettuce as Impacted by Soil Contamination Level

– 3 sets of 25g 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 Lettuce			2nd Lettuce			3rd Lettuce		
			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	ND	ND	ND
	2	22000	9.1	6.5	99	2.1	1.6	1.6	ND	ND	ND
	3	25000	4.6	9.1	140	0.89	1.6	2.7	ND	ND	ND
10^3-10^4	1	4200	0.89	0.8	0.4	ND	ND	ND	ND	ND	ND
	2	3800	0.4	0.89	0.4	ND	ND	ND	ND	ND	ND
	3	3600	ND	0.89	0.8	ND	ND	ND	ND	ND	ND
10^2-10^3	1	380	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	380	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	240	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^1-10^2	1	28	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	40	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	32	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^0-10^1	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND$^{£}$- ND$^{£}$-*Not detectable at a detection limit of 0.36 MPN /g lettuce.*
Soil: Salinas Sandy-loam; 10% moisture.

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Table 3. Cutting Blade-assisted *E. coli* O157:H7 Transference to Lettuce as Impacted by Soil Contamination Level

– 100g samples from the cored region

Target inocula (MPN /g Soil)	Actual inocula (MPN /g Soil)	*E.coli* O157:H7 Recovered on Lettuce (MPN /g lettuce)								
		1st Head			2nd Head			3rd Head		
		Replication			Replication			Replication		
		1	2	3	1	2	3	1	2	3
10^5-10^6	430000	52	15	1.5	2.1	1.1	ND	ND	ND	ND
10^4-10^5	21000	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^3-10^4	1200	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^2-10^3	310	ND	ND	ND	ND	ND	ND	ND	ND	ND
10^1-10^2	58	ND	ND	ND	ND	ND	ND	ND	ND	ND

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Table 4. Cutting Blade-assisted *E. coli* O157:H7 Transference to Lettuce as Impacted by the Contact Area between Blade and Lettuce

- 100g samples from the cored region

Blade to Lettuce Contact	***E.coli* O157:H7 Recovered on Lettuce (MPN/g lettuce)**								
	1st Head			2nd Head			3rd Head		
	Replication			Replication			Replication		
	1	2	3	1	2	3	1	2	3
A. Stem only	ND	ND	ND	ND	ND	ND	ND	ND	ND
B. Some contact	2.3	1.7	3.3	0.68	0.2	0.8	ND	ND	ND
C. Moderate contact	230	330	130	49	110	33	1.3	17	2.3

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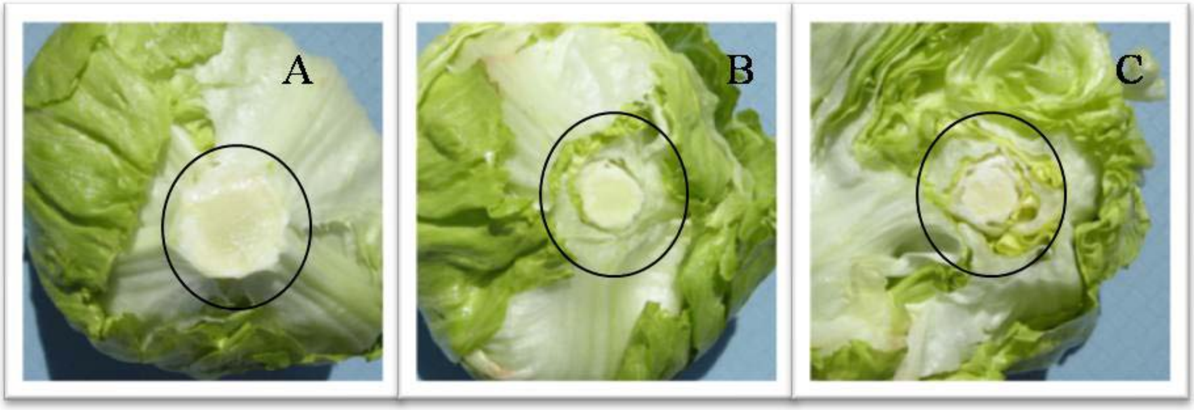

Figure 4. Blade to lettuce contact. A. Blade contacted the stem only; B: Blade contacted stem and small amount of our lettuce tissues that remained on the harvested lettuce after core removal; C: Blade contacted the stem and a significant amount of lettuce tissues remained on the harvested lettuce after core removal. Lettuce tissues located within the circles were removed during core removal.

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Table 5. Blade-assisted *E.coli* O157:H7 Transference to Lettuce as Impacted by Soil Moisture level
– 100g samples taken from the cored region

Soil moisture	Actual inocula (MPN /g Soil)	Replication	Soil amount (g/knife)	1st Head	2nd Head	3rd Head
30%	260,000	1	30.13	1300	13	3.3
		2	32.02	2300	23	13
		3	27.12	230	3.3	1.7
25%	430,000	1	23.9	78	0.78	ND
		2	20.7	13	2.3	ND
		3	22.6	4.5	ND	MD
20%	160,000	1	9.9	ND	ND	ND
		2	9.3	ND	ND	ND
		3	10.9	ND	ND	ND
15%	260,000	1	0.72	ND	ND	ND
		2	0.81	ND	ND	ND
		3	1.02	ND	ND	ND
10%	110,000	1	0.05	ND	ND	ND
		2	0.06	ND	ND	ND
		3	0.04	ND	ND	ND

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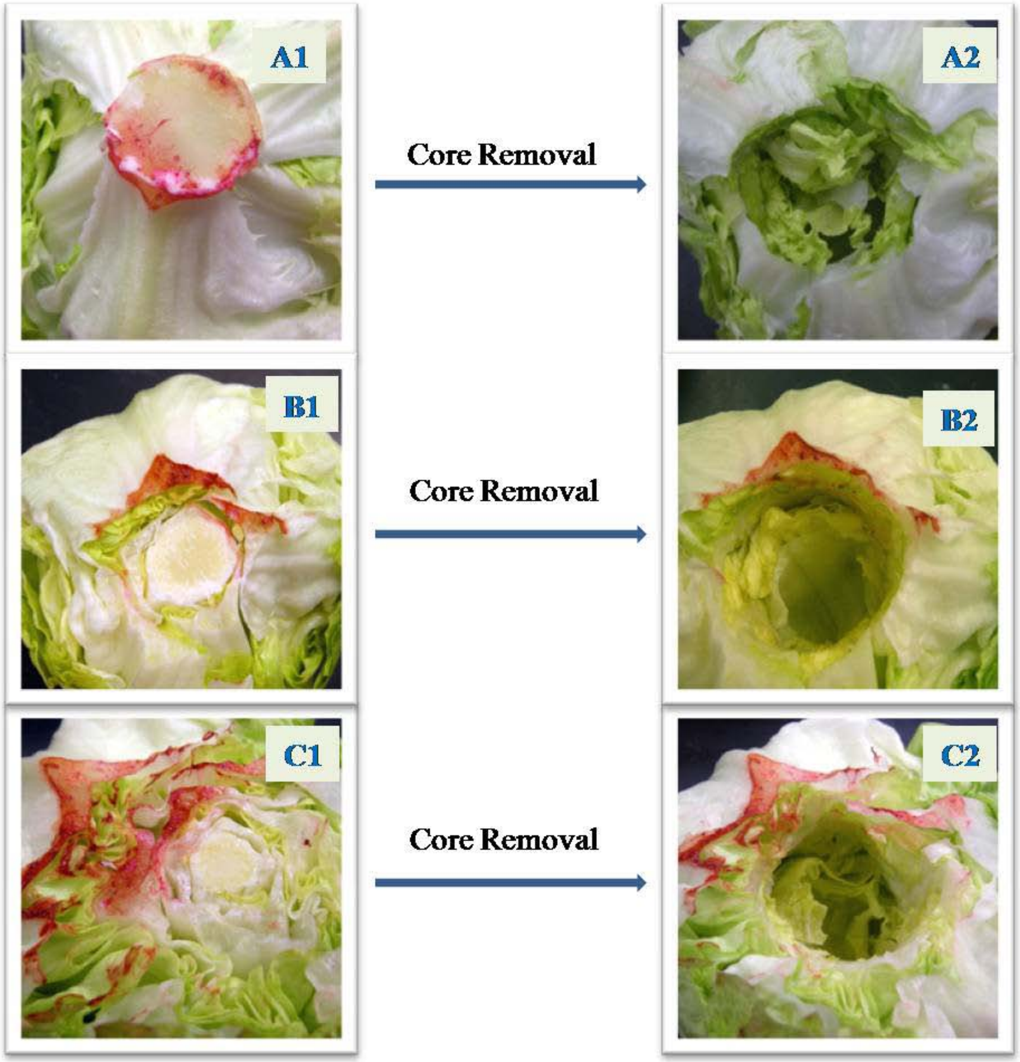

Figure 5. The impact of blade-to-lettuce contact on the transference of contaminants to the harvested lettuce. The blade was stained with red dye.

A. When the blade only contacts the stem (the waste), the contaminants will likely be removed during core removal.
B. When blade contacts both the stem and some amount of lettuce tissues located outside of the coring path, contaminants will remain on the harvested lettuce,
C. When blade contacts an extensive amount of lettuce tissue located outside the ring path, the majority of the contaminants will remain on the harvested lettuce.

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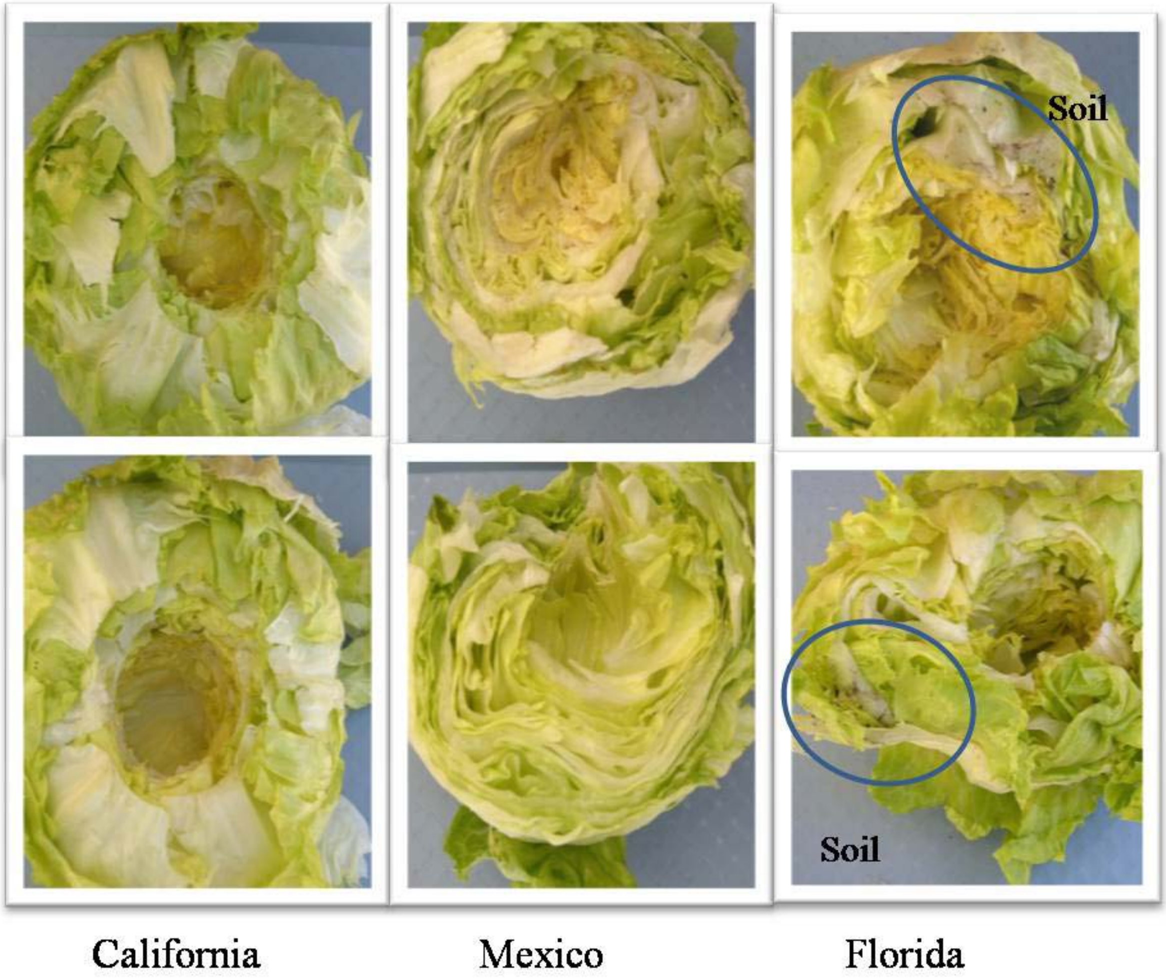

Figure 6. Figure 6. Photos of CIF lettuce harvested from different regions. Note that samples from California appeared to be cut from the plant with a cutting blade, and cored with a coring ring. Samples from Mexico appeared to have a significant amount of direct contact between the cutting blade and the edible lettuce-head tissues. Samples from Florida have soil present on both cut/cored regions, and the other non-cut regions.

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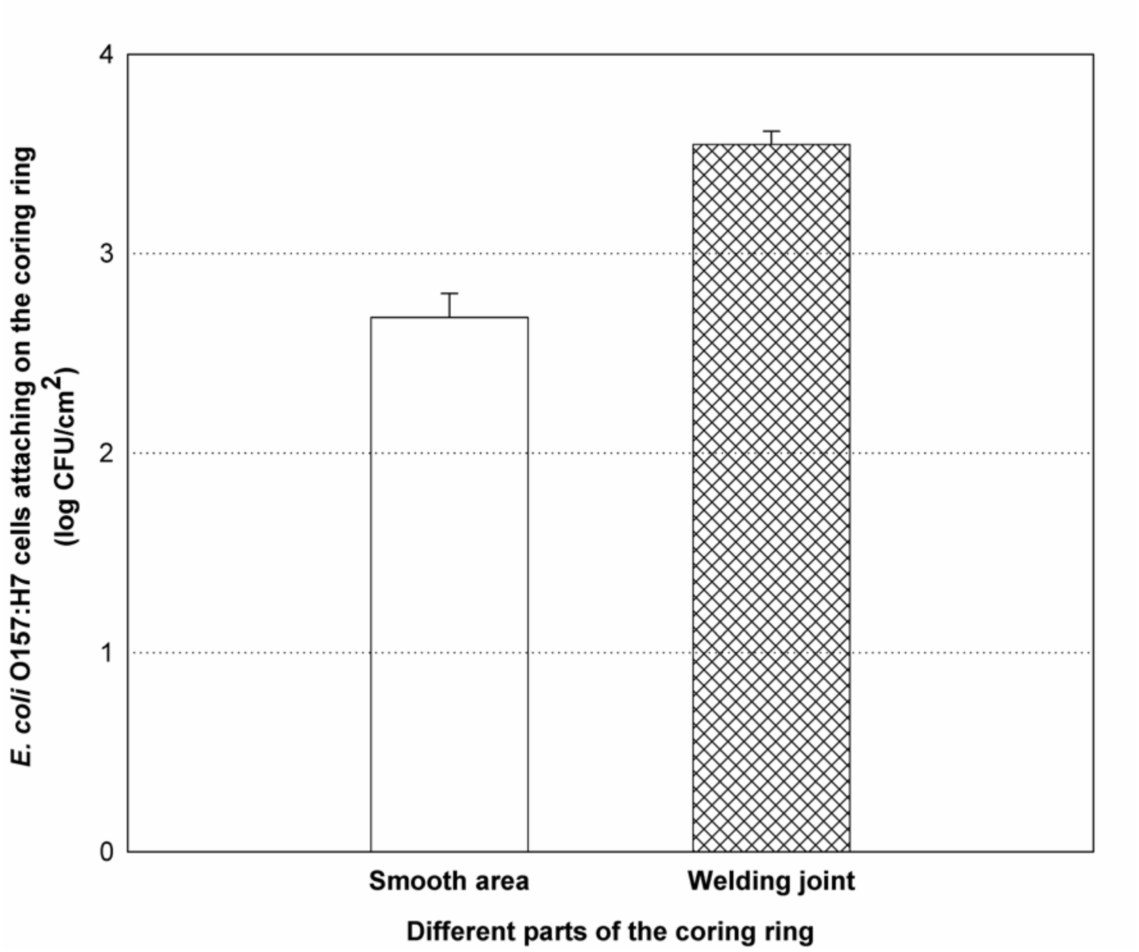

Figure 7. Attachment of *E. coli* O157:H7 on the smooth and rough welded joint of a typical lettuce coring knife.

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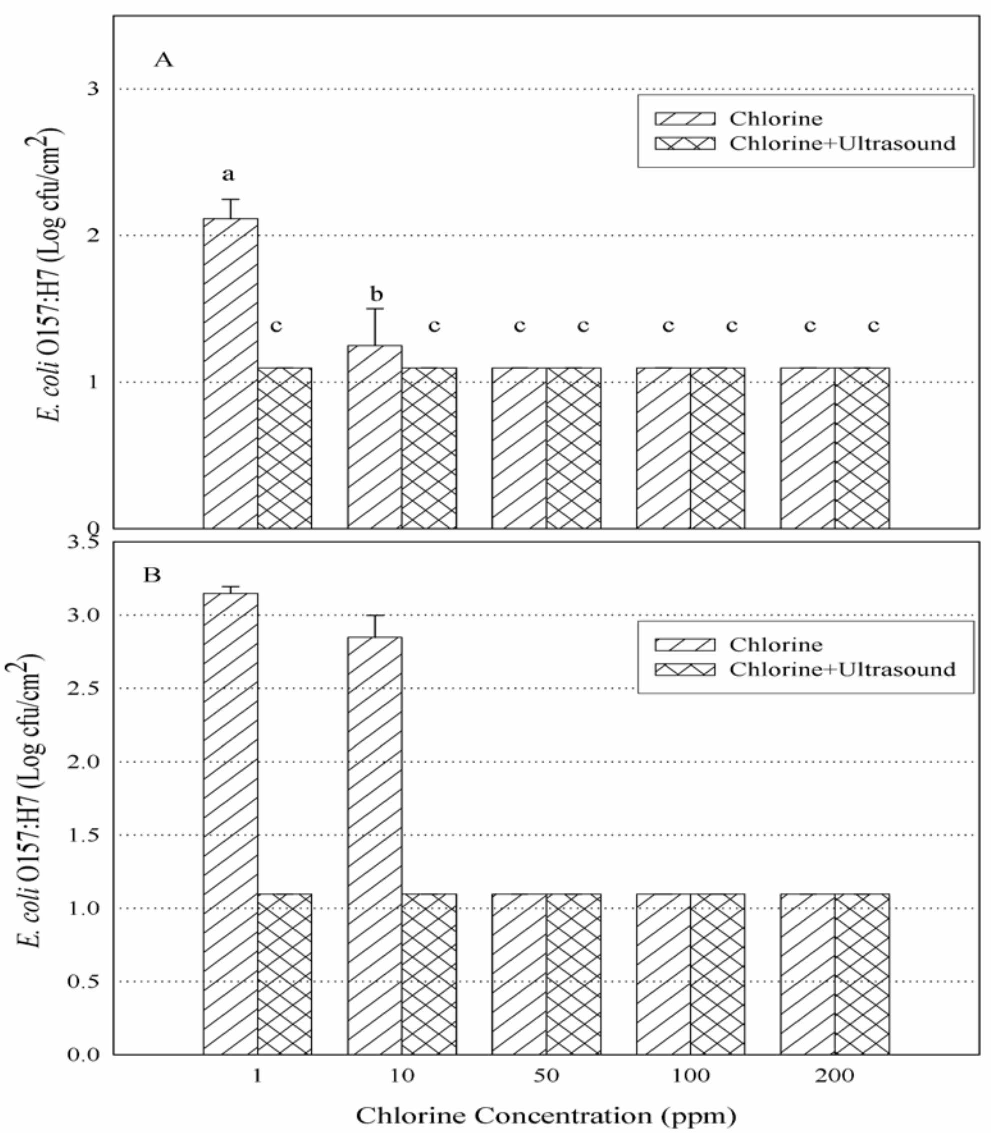

Figure 8. Recovery of *E. coli* O157:H7 on the smooth (A) and rough welded point (B) of a typical coring knife treated with chlorine alone or in combination with ultrasound for 1 min. ND: Non-detectable level

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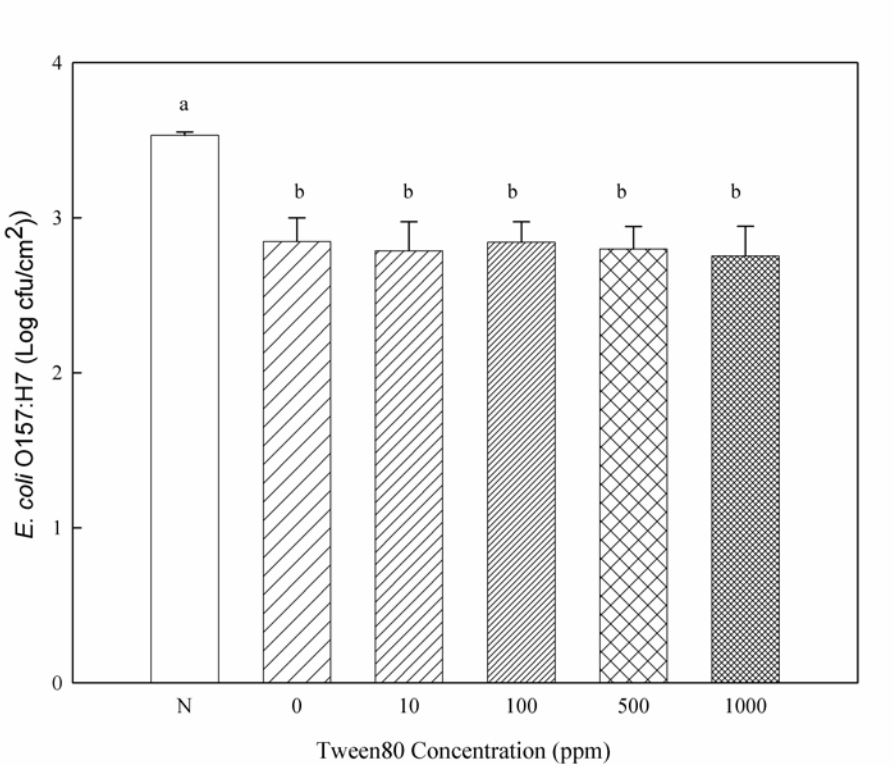

Figure 9. Recovery of *E. coli* O157:H7 on the rough welded joint of a typical lettuce coring knife treated with 10 ppm chlorine alone or in combination with Tween80 for 1 min. N: Un-treated control.

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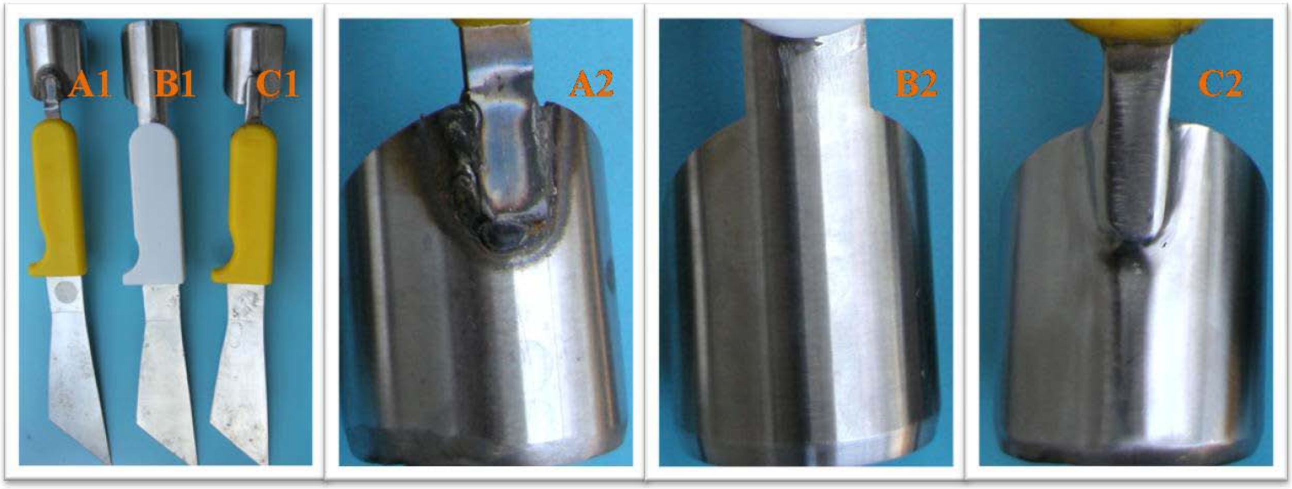

Figure 10. Development of prototype coring knife with improved food safety feature.
A1: Coring knife currently widely used in the industry. A2: Enlarged image showing the rough welding of the coring ring
B1: Prototype 1 – No-joint (one piece) design; B2: enlarged image showing no welding point
C1: Prototype 2. Current coring knife with rough welding point polished; C2: enlarged image showing the smooth welding pint.

Figure 11. A field test was conducted in California comparing the newly designed corning knife with one currently used in the lettuce coring operation. An operator showed the two knifes after coring lettuce heads.

Left: Commercial CIF harvest knife
Right: Prototype CIF harvest knife with no-joint

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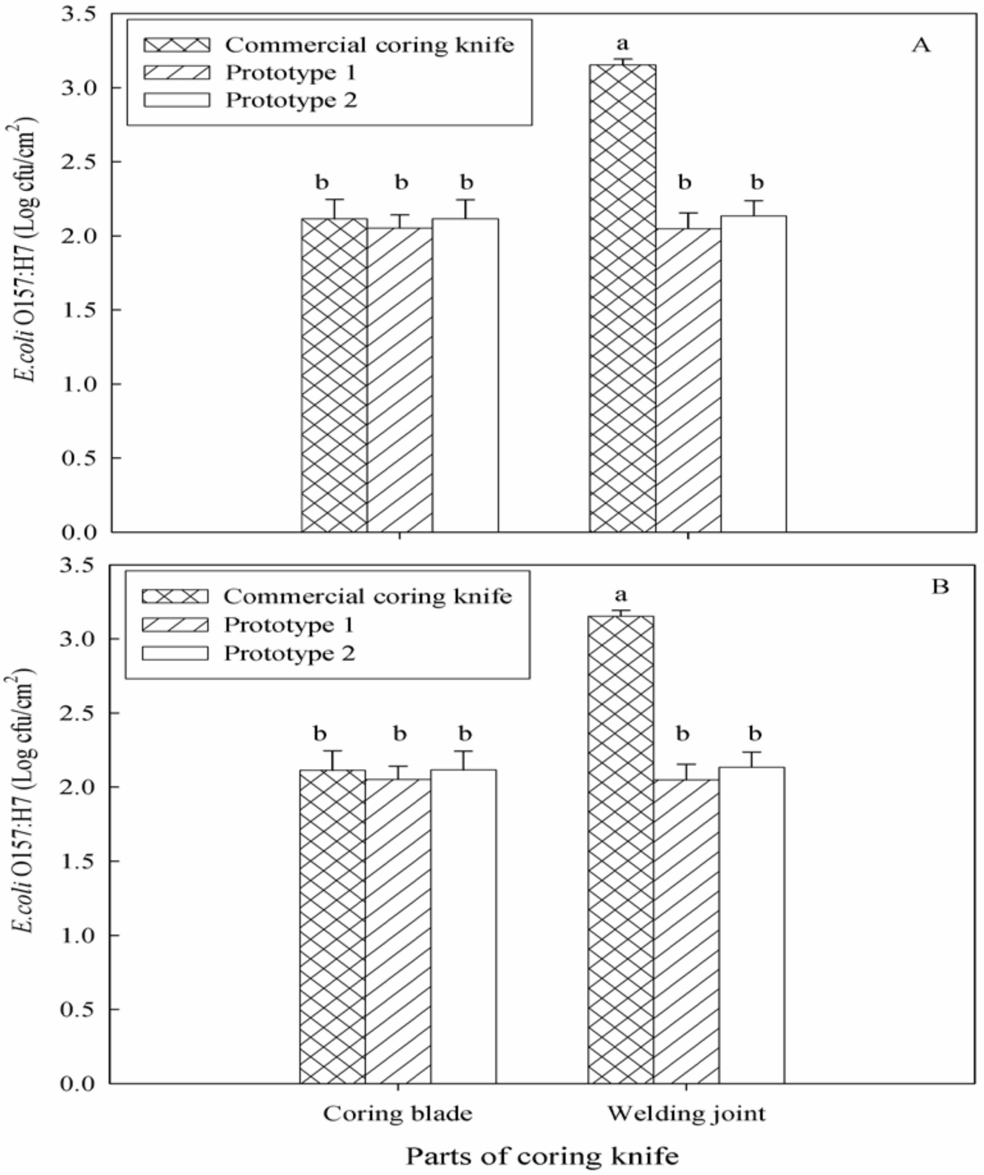

Figure 12. The attachment (A) and removal/inactivation (B) of *E. coli* O157:H7 on the currently used coring knife, and the two prototypes with rough welding point removed or smoothed. Chlorine concentration: 10 ppm.

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Appendix I

Ultrasound as an Intervention Technology for the Sanitation of Coring Knife

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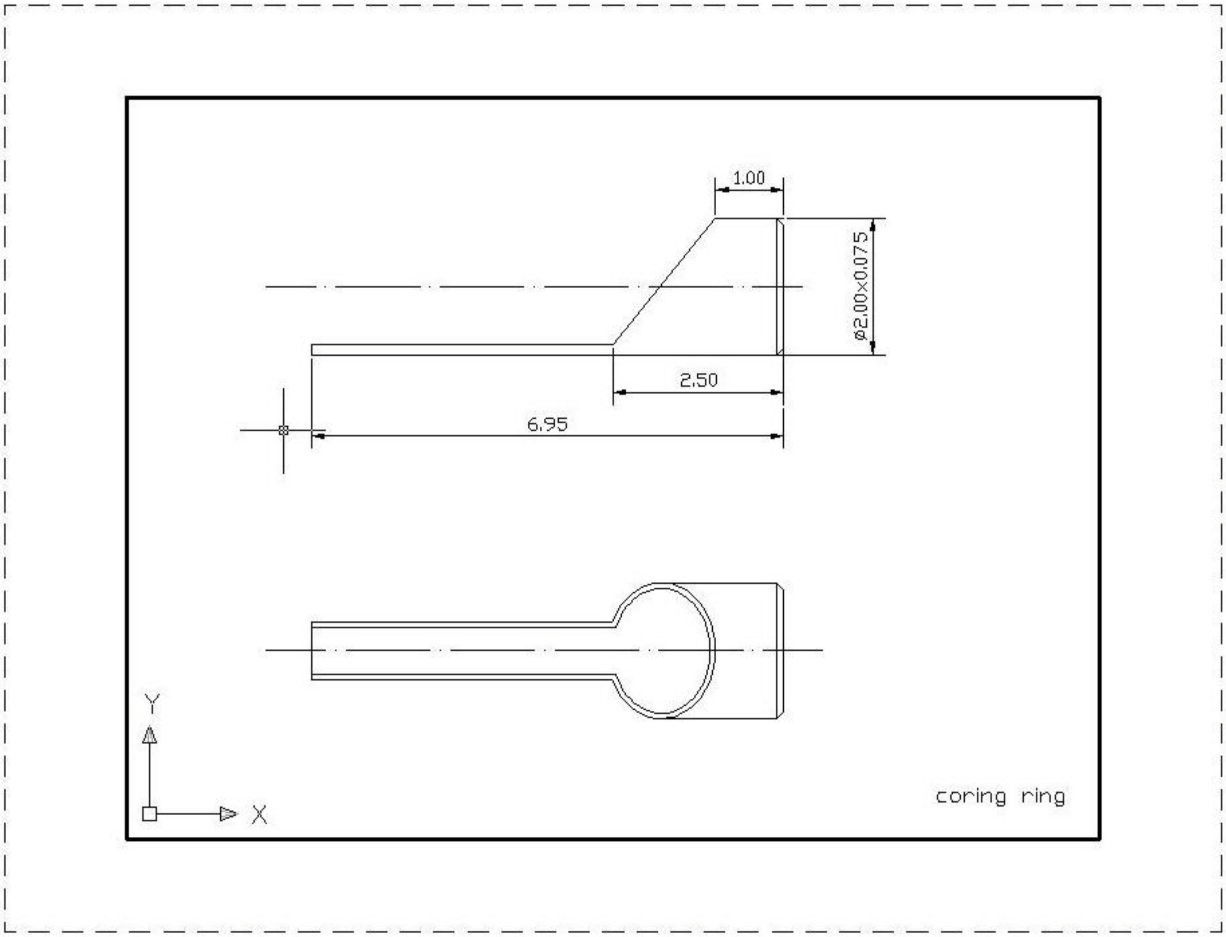
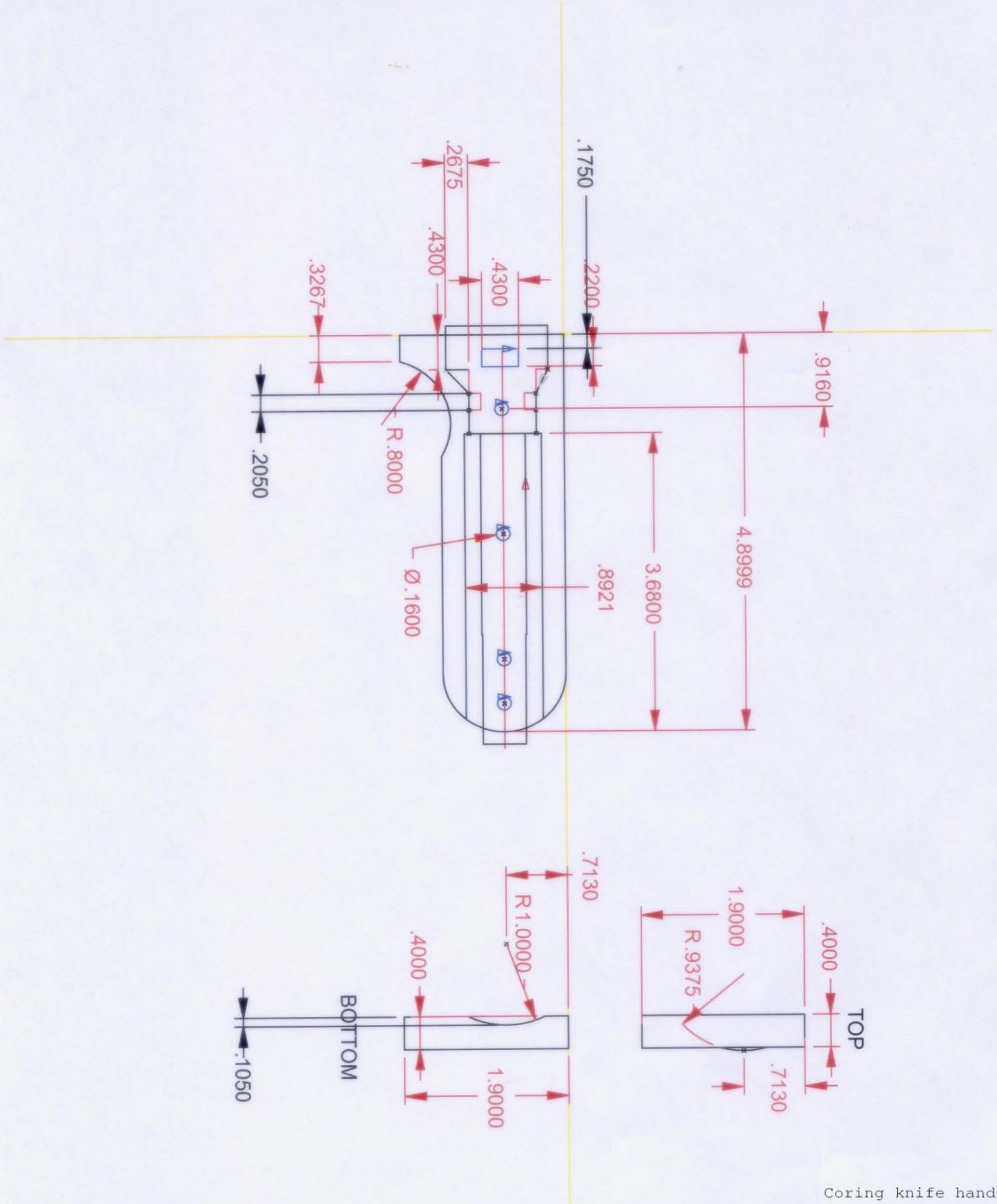
Abstract: Lettuce field coring and trimming practice is a recent industry development designed to improve fresh-cut processing yield and reduce shipping costs. Studies showed that the harvesting knives used could be potentially contaminated with pathogens via contact with contaminated soils and plants, and thus serve as vehicles for subsequent contamination of the harvested lettuce. Chlorine is currently used to sanitize the harvesting knives with limited efficacy on pathogen inactivation. Ultrasound has demonstrated effectiveness for sanitizing the food contact surfaces of stainless steel equipment, and yet has not been tested for its potential for sanitizing lettuce harvesting devices such as corning knives. This research examined the effect of ultrasonication and chlorine on the reduction of *E. coli* populations on coring knives. The knife was inoculated by dipping in soil slurries containing *E. coli* cells for 1 minute followed by air-drying for 2.0 hours. The knife was then submerged in the solutions containing 1, 10, 50, 100, or 200 ppm free chlorine, with or without ultrasonication (25 kHz, 500W/L) for 0.5, 1, or 2 min. The surface of the knife was swabbed with wetted cotton rods and enumerated for *E.coli* populations via a direct platting method. Results indicate that the rough welding point of the knife harbored more *E.coli* cells than the smooth areas. An ultrasonication treatment significantly improved the efficacy of chlorine on *E.coli* inactivation on coring knife, especially on the welding point. A 30-second treatment of ultrasonication (25 kHz, 500 W/L) and 1 ppm chlorine treatment reduced the *E. coli* population to the non-detectable level, whereas a significant level of *E.coli* cells remained when the knife was treated with chlorine alone. This suggests that ultrasonication holds a great potential for improving the sanitation of lettuce harvesting device.

Appendix 2

Drawings of the parts of the prototype 1 coring knife

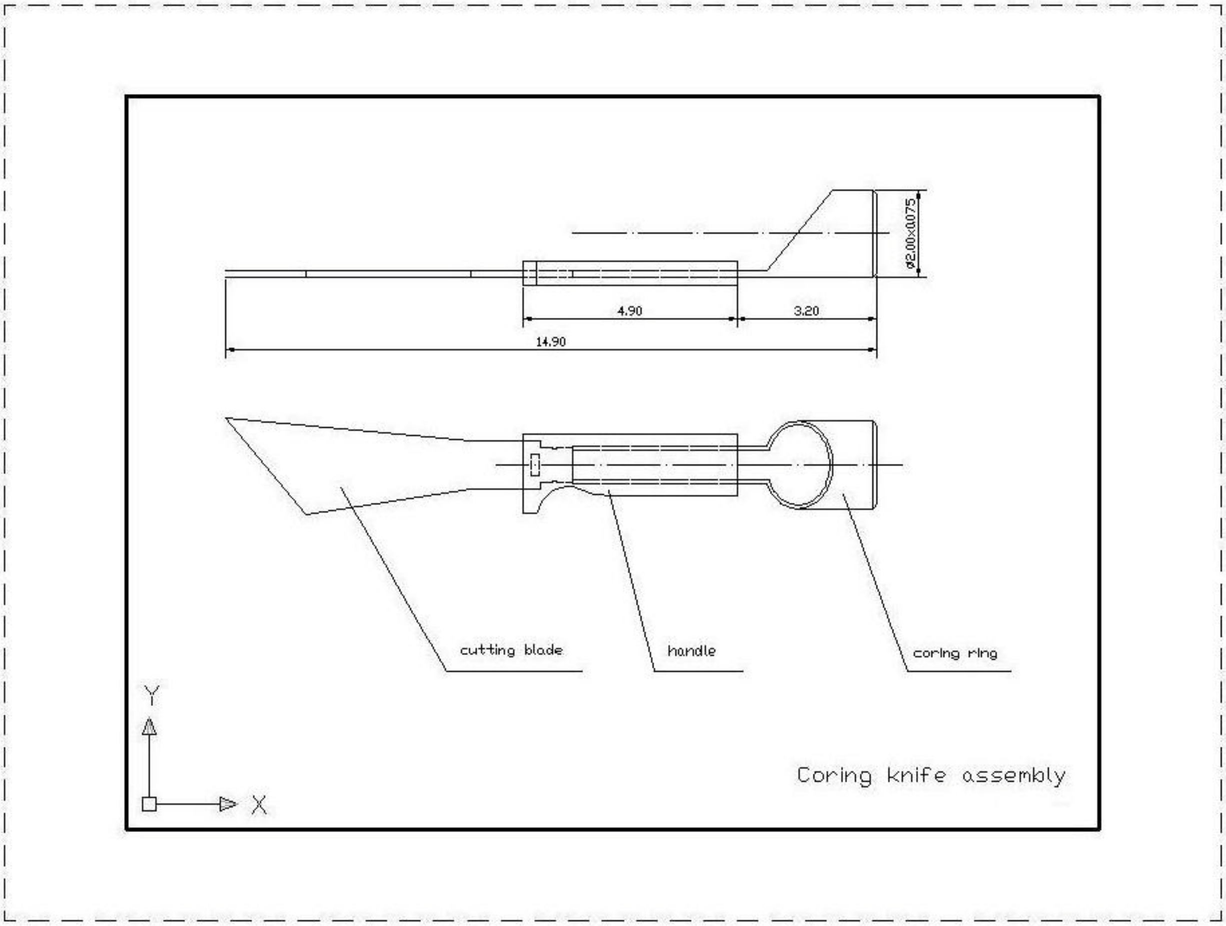


Drawing 1 Coring ring



Coring knife handle

Drawing 2 Coring knife handle



Drawing 3 Coring knife assembly