Center for Produce Safety STEC Seasonality Project:
Romaine Lettuce Seasonal Risk in the California Central Coast Region
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CPS STEC Issue Brief 1: Hypothesis Risk Matrix

Summary Statement: Throughout the project, a concerted effort was made to identify sources of practical and scientifically developed data, facts, and expertise. The aim was to both identify and encourage cooperative approaches to align and analyze the full range of causal hypotheses in the search for root cause(s) identification and solutions to recurring seasonal outbreaks. The initial objective was to address the broad concerns and questions surrounding Shiga toxin-producing *E. coli* (STEC) sources and contamination within the California Central Coast region, with a focus on the reoccurring

E. coli O157:H7 outbreak subtype and romaine lettuce. From the outset, it was clear that a priority area was to assemble and align facts and data for outbreaks where recurring outbreak subtypes were unequivocally detected on commercially distributed romaine lettuce. This foundation was viewed as essential to support subject matter expert analysis in ruling in or ruling out any given hypothesis brought forward by diverse stakeholders. Despite substantial effort and sound progress, these insights to hypothesis risk ranking and risk exposure never fully materialized. Therefore, we could not make any specific delineation between hypothesis generation and fact- or data-supported applicability to production, harvest, shipping, processing, or distribution. The outcome from the collective effort is a catalogue list of the multiple root-cause possibilities. Hopefully, this document and the companion Issue Briefs will find use as a resource for future industry actions and identification of research questions and priorities.

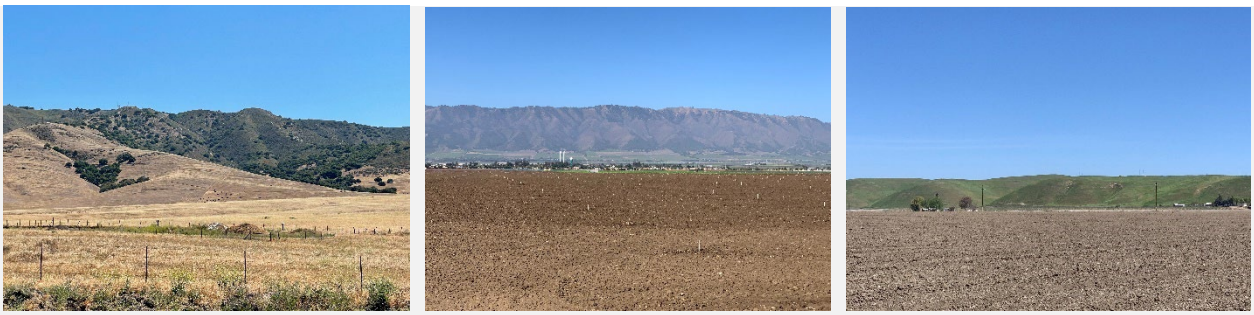

Figure 1. Three views of the regional landscape from the perspective of leafy greens production sites to rangeland cattle management locations. The leading hypothesis generation questions include: "Are the recurring STEC isolates always around or seasonally re-introduced?" "Does seasonal management of cattle movement, in combination with seasonal romaine growing locations and practices, align with outbreak timelines and specific product lot-source data?"

Purpose: This document categorizes the hypothesis generation concepts developed from largely long-standing concepts surrounding discussions of seasonal romaine lettuce outbreak root cause. An effort to assign risk ranking to each hypothesis factor and risk-influencing co-factors, based on subject matter experts and informed practitioners, is included in the matrix tables below. Several hypothesis generation concepts include new sub-parts which, while part of prior more limited discussions, become more openly developed within the timeline of this project. Throughout the duration of this project many discussions and on-site/on-farm visits were conducted by the Project Lead and diverse engaged interest groups. The suggested risk ranking within the tables is largely the

synthesis and consolidation of these inputs by the Project Lead. These rankings are not an authoritative or final analysis of each hypothesis factor but can serve as a catalyst for continued dialogue and concept refinement towards research needs and priorities. The anticipated outcome of this series of tables is to further the dialogue among diverse stakeholders in prioritizing more immediate prevention and mitigation protocols and in refining research needs and opportunities.

Definition: For this project, hypothesis generation towards focus and prioritization of root cause analysis is described as follows:

- applying available knowledge to assemble all plausible factors that may explain the root of a problem rather than the outcome, and
- aligning diverse science-based and practical knowledge, facts, and specific data to identity the why of the problem (recurring and consequential seasonal product contamination) and not the what (recurring regional outbreaks), and
- then employing the root cause analysis process to evaluate each hypothesis and use the above information, product-specific data, and subject matter expert discussions to **rule in** or **rule out** a hypothesis for a specific implicated lot and, more broadly, for a practice or a region.

How to Use This Root Cause Hypothesis Matrix

The 10 matrix tables provided below may be useful to readers for general understanding of the complexity and interconnectivity of hazards, risk, and predicating influences or co-factors on product contamination and subsequent persistence and potential growth. All potential risks and commercial practices and environmental factors included are known to exist and are possibly involved in the seasonal outbreaks of *E. coli* O157:H7 within the California Central Coast romaine lettuce production region; however, not all may be factual and true for a specific incident or location. The matrix tables are intended only as a guide and not as a definitive set or the only interpretation of priorities towards a root cause determination and future preventive or mitigation solutions. Other readers may find the line items and assembled characterization of risk factors helpful in developing research priorities and experimental design concepts.

Key to Matrix Tables

This Issue Brief provides a more inclusive set of hypothesis generation concepts relevant to STEC seasonality for the Central Coast of California than what is known to be factual

for an individual outbreak-implicated locale or farm of interest. The rationale for this approach is that subject matter expert discussions involved many logical hazards and risk factors that might be aligned with root cause when trying to rule in or rule out any component hypothesis at the regional level as well as any that might apply to a specific product lot identified in outbreak traceback.

Ranking Guide Scheme –

Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Descriptors	High	Likely	Descriptors	High
Descriptors	Moderate	Uncertain	Descriptors	Moderate
Descriptors	Low	Unlikely	Descriptors	Low

Hypothesis Risk Ranking – An informed opinion on the likelihood of a hypothesis fitting a specific root cause scenario.

- High (highly likely, supported by fact or data)
- Moderate (possible broadly but lacking facts of data to make a priority)
- Low (facts or data rule out for locale or farm-specific outbreak root cause)

Current Risk Rank Probability – An informed opinion on the likelihood that the hypothesis-related risk has merit to be retained in root cause analysis fact-gathering and/or directed research priorities to fill knowledge gaps for STEC Seasonality for the CA Central Coast.

- Likely (good to strong hypothesis association with risk)
- Uncertain (no current evidence to support hypothesis but remains plausible)
- Unlikely (good to strong evidence to exclude hypothesis association risk factor)

Key Co-Factors Modulating Risk Profile – For almost any risk-defining hypothesis, a single factor fully explains the root cause only in an exceptional situation. The key co-factors influencing risk potential (e.g., *E. coli* O157:H7 growth) or risk exposure (e.g., hillside run-off or flooding events at a production lot) are listed that relate to Risk Rank Probability determination.

Research Priority Rank – An informed opinion intended to provide guidance on research topic prioritization. At this time, no distinction is drawn between research topic priorities for CPS or other potential longer-term funding sources.

- High (research highly likely to provide solutions or enabling knowledge)
- Moderate (research highly likely to result in knowledge gap-filling or long-term solution)
- Low (researchable question unlikely to be defined, meaningful, or implemented)

Figure 2. In the focus area for recent outbreaks, regional run-off during peak winter rains (January-March) flows from hillsides towards leafy greens production, and the last chance for rainfall is typically by April 10. Evidence of fecal matter transport in these dry washes following seasonal storm events may be observed. Seasonal flooding within the broad region is known, but there is no evidence for such events from 2017-2020 in the implicated production areas. In addition, the seasonal production timing occurs during the driest months of July-September, which raises many questions regarding STEC dispersal, transmission, vectors, and reservoirs of the specific reoccurring *E. coli* O157:H7 subtype.

Hypothesis Generation Risk Factor and Co-Factor Components of Root Cause Analysis

SOIL

SOIL Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Stealth reservoir	Moderate	Unlikely	Amendments, crop residue, rotation crop	Moderate

SOIL Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Amplification site	Moderate	Uncertain	Amendments, crop residue, rotation crop	High
Dependent Risk Factors				
Soil type and texture	High	Likely	Soil organic matter content, silt-clay content	Low
Irrigation water characteristics /analysis	Moderate	Uncertain	Season weather; animal/bird intrusion; treatment performance	Moderate
Seasonal PS and NPS run-off sources	Low	Unlikely	Run-off, flooding not excluded at specific farm site; transfer from adjacent crop or non-crop areas	Low
Preceding rotation crop	High	Likely	Crop management practices; crop cycles per season – knowledge gap area	High
Historic crop rotation	Moderate	Uncertain	Crop management; soil texture – knowledge gap area	Moderate
Crop residue biomass	High	Likely	Seasonal weather; crop specific; in-season amendments; animal intrusion	High
Organic amendments	Moderate	Unlikely	Facts and data implicating organic management in outbreak traceback lot	Low
Adjacent crop soil and run-off	Low	Uncertain	Facts and data indicating failure to control in outbreak traceback lot	Low
In-season soil transfer to crop	Moderate	Likely	Facts and data implicating high transfer rates or high contaminated transfer in positive lot or outbreak traceback lot; cultivation practices; irrigation conveyance	High

SOIL Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
			and application movement in crop season	
At harvest soil transfer to product	High	Uncertain	Harvest practices; field conditions; product quality and trimming	Moderate
At harvest soil transfer to equipment, workers, and implements	High	Likely	Harvest practices; field conditions; product quality and trimming; market supply and demand market factors; equipment design, management, cleaning and sanitation	Low if requiring commercial site access and testing
				High if model research challenge study or known contaminated crop is secured

Figure 3. Hypothesis generation around the potential root cause co-factors of crop rotation and immediately adjacent crops has many interacting components, including soil organic matter, soil and fertility management inputs, differential wildlife attraction and grower tolerance, and movement of water conveyance and application equipment during cultivation events. Broccoli and romaine are a frequent contemporary or sequential pair.

WATER

WATER Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Resident presence of STEC in water source	High	Unlikely	Sediments; algae; position of outlet suction (contact or near sediment layer)	Moderate (known factor)
Seasonal increase in STEC	High	Uncertain	Surface reservoir; shallow well; algae; birds; water fowl; bioaerosols	Moderate (mostly known factors)
Conveyance amplification	Moderate	Uncertain	Seasonal temperatures; fertigation amendments	Moderate (known factor)
Conveyance movement crop/field	Moderate	Uncertain	Soil uptake to conveyance; soil amendments; flooding, prior crop; crop residue amplification of STEC	Low – requiring commercial site access and testing
Treatment failures	High	Likely	STEC uptake from source to system; protection in suspended sediments; protection in micro-algal fragments; protection in protists	Moderate (mostly known factors)
Crop treatment (foliar sprays; fertigation) equipment chronic or acute contamination	Moderate	Uncertain	Ineffective cleaning and sanitation; use of untreated surface water; crop materials allow amplification; warm to hot seasonal temperatures	Moderate (mostly known factors)
Crop treatment make-up source water (foliar sprays; fertigation)	High	Likely – depends on water source Uncertain – depends on materials and environmental	Use of untreated surface water; crop materials allow amplification; warm to hot seasonal temperatures; hold time in tanks	Moderate

WATER Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Crop treatment additive amplification	High	Uncertain	Use of untreated surface water; crop materials allow amplification; warm to hot seasonal temperatures; hold time in tanks	Low due to mostly known risk factors, and specific study would likely require commercial site access and testing

Figure 4. Ground water pumped to on-farm reservoirs is one source of regional crop production water. Irrigation canals are not used in this region, as compared to other major western production regions. Direct extraction of river water for crop production is not practiced, as an over-arching rule, and no evidence for this practice was identified in outbreak or recall implicated areas during this project. Late season risk factors, including macro-algae growth, sediment suspension during high winds, and increased bird or water fowl presence due to high temperatures, are recognized. Treatment of this surface water has been a practice on many farms during the relevant period. Hypothesis generation and risk ranking identifies water treatment efficacy under these conditions as a key knowledge gap.

CROP

CROP Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Variety	Low	Uncertain	Growth habit/openness; heat tolerance/ seasonal planting slot	Moderate – no current facts or evidence that outbreaks have been variety influenced
Varietal geographic distribution	Moderate	Uncertain	Seasonal/regional planting slot	Moderate – no current facts or evidence that outbreaks have been variety-use influenced… granularity of industry data not available
Varietal influence on microbiome	High	Likely	Romaine varieties influence microbiome; locale; seasonal environmental conditions; crop management practices; foliar treatments; site-specific soil microbiome	Moderate – long-term research and high uncertainty as to specific interactions and relation to outbreaks and positive preharvest test outcomes; industry data to optimize objectives, experiment design; assurances of specific access to sites not available
Irrigation mode	High	Uncertain	Overhead vs. drip; drip applied in manner to create furrow pooling or flooding; surface water source not treated during critical	Moderate – many known factors but uncertainty as to specific interactions and relation to

CROP Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
			STEC exposure timing; surface water not adequately treated during critical STEC exposure timing	outbreaks and positive preharvest test outcomes; industry data to optimize objectives, experiment design; assurances of specific access to sites not available
Irrigation conveyance movement and use practices	Low	Uncertain	See SOIL and WATER tables; placement of in-use conveyance during cultivation; back-flow contamination	Low due to mostly known risk factors, and specific study would likely require commercial site access and testing
Romaine head architecture	Moderate	Uncertain	See Variety, but main focus is on open heading structure; influenced by variety and location; seasonal temperatures as an elevating factor in openness near maturity	Moderate – some known or associative factors but uncertainty as to specific interactions and relation to outbreaks and positive preharvest test outcomes; assurances of specific access to multiple assessment sites not available
Cultivation practices	Moderate	Likely	See SOIL table; STEC in soil as a chronic reservoir or acute contamination source; stage of growth; soil type; speed of tractor during cultivation	High as a research objective in model studies but uncertainty as to linkage to natural contamination events

CROP Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Seasonal growth rate	Moderate	Uncertain	See WATER table and Varietal influence on microbiome; changes in leaf surface topography and physicochemical properties	High as a research objective in model studies but uncertainty as to linkage to natural contamination events
Seasonal non-market leaves defects/ trimming	High	Likely	Increased injury/defect sites for contamination survival; cross-contact transfer of contamination during harvest operations	High as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events
Intended market use	Moderate	Uncertain	Degree of trimming and handling practices at harvest	
Adjacent crop type and practices – same grower	Moderate	Uncertain	Site specific crop management, inputs, cross-crop interactions, system equipment practices	High as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events
Adjacent crop type and practices – different grower	Moderate	Uncertain	Site specific crop management, inputs, cross-crop interactions, system equipment practices	High as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events


Figure 5. A long-standing explanation for the question "Why Romaine?" has been the upright and open-heading structure during development, offering protection and survivability for pathogens in protected sites. During this project period, walking the rows during and immediately following cultivation events we identified soil aggregates in the central whorl as a hypothetical risk factor. With overhead irrigation these aggregates are rarely observed during harvest but may have carried contaminants to crop tissue, which may become internalized during maturation and tighter columnar head formation. Romaine varieties have different open-heading characteristics and this is further influenced by environmental conditions. Openness at maturity is generally a trait during higher growth temperatures and rates. The prevalence was observed infrequently within some fields and prevalent in others during 2020.

HARVEST

HARVEST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Field preparations	Moderate	Low	See SOIL table	Low – specific study would likely require commercial site access and testing
Equipment staging	Moderate	Uncertain	See DOMESTICATED ANIMAL SOURCES table; see BIOAEROSOLS table; see WILDLIFE AND INSECTS; See CROP table – Adjacent crop	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing
Packing/bin staging	Low	Uncertain		Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing
Equipment design and fabrications	High	Likely	Factors related to site, prior harvest sites, cleaning and sanitation performance	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing

HARVEST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Cleaning and sanitation timing	High	Uncertain	Factors related to seasonal conditions and duration between harvest-stop and cleaning event; amplification sites	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing
Equipment lot-to-lot movement	Moderate	Uncertain	Effectiveness of cleaning and sanitation	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing
Equipment field-to-field movement	Same as Equipment lot-to-lot movement			
Tractor lot-to-lot movement	Same as Equipment lot-to-lot movement			
Tractor field-to-field movement	Same as Equipment lot-to-lot movement			
Equipment sub-unit movement grower-to-grower region-to-region	Same as Equipment lot-to-lot movement			

HARVEST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Crew lot-to-lot movement	Moderate	Uncertain	Food safety systems performance	Low
Crew field-to-field movement	Moderate	Uncertain	Food safety systems performance	Low
Crew region-to-region movement	Moderate	Uncertain	Food safety systems performance	Low

Figure 6. Hygienic design and enhanced cleaning and sanitation practices for harvest implements and mobile equipment has been an industry focus during this project period. Alignment of locations and timelines for this harvest-aide equipment is relevant to both the broad and implicated lot root cause analysis.

POSTHARVEST

POSTHARVEST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Cut-to-cool timing	Moderate	Uncertain	Presence of contaminated product; time of harvest; time of harvest cut-off at high air temperatures	Moderate (some evidence from research known) – no current facts or evidence specific to outbreaks or preharvest or finished product test results… granularity of industry data not available
Cut-to-cool temperatures	High	Likely	Presence of contaminated product; warm to high temperature; duration of cut-to-cool timing	Moderate (some evidence from research known) – no current facts or evidence specific to outbreaks or preharvest or finished product test results… granularity of industry data not available
Source processing	Moderate	Likely	Presence of contaminated product; quantitative contamination levels on raw product; performance of postharvest and processing food safety systems	Under evaluation by CPS
Forward processing	Moderate	Likely	Presence of contaminated product; changes in STEC physiology/stress recovery during pre-processing intervals	Under evaluation by CPS

POSTHARVEST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
			and cold chain management; quantitative contamination levels on raw product; raw product temperatures experienced in transport; performance of postharvest and processing food safety systems	
Transportation	Moderate	Likely	Cold chain performance	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing
Distribution	Moderate	Likely	Cold chain performance	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing


Figure 7. Current facts and evidence point to preharvest or harvest factors as sensible points of focus to prevent initial contamination, but postharvest handling and processing remain a concern for growth or for recovery conditions for sub-lethally injured or stressed cells. Research has provided evidence that these surviving bacteria may be induced to increased virulence (infectivity) in the postharvest environment, even if growth is not possible.

DOMESTICATED ANIMAL SOURCES

ANIMAL Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Cattle proximity	High	Likely	See BIOAEROSOLS table; see WILDLIFE AND INSECTS table; human vector potential (mechanical transfer, shared road traffic); diverse vector presence and activities; proximity of animal congregation sites	
Cattle movement	High	Likely	As above; see EVOLUTIONARY GENOMICS table for specific recurring strains risk root cause; seasonality of interstate and intrastate movement	
Corrals and transfer stations	High	Uncertain	As above for proximity and movement	
Rangeland vehicle traffic	Low	Uncertain	As above; non-cattle management vehicles in rangeland entering leafy greens production areas	Moderate as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing to acquire quantitative transfer potential

ANIMAL Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Shared farm road traffic	Low	Uncertain	As above; non-cattle management vehicles in rangeland entering leafy greens production areas	Low as a research objective in model studies (some known as risk factor) but uncertainty as to linkage to natural contamination events: specific study would likely require commercial site access and testing to acquire quantitative transfer potential

Figure 8. Current hypotheses for dispersal of STEC and the recurring *E. coli* O157:H7 subtype have centralized around cattle congregation sites such as transfer corrals, water troughs, supplemental feeding stations, and shaded areas. The primary rationale is connected to the greater concentration of manure deposits and, therefore, STEC abundance, with activity more likely to create mechanisms of dispersal. Potential vectors, including birds and feral swine with long-range foraging behaviors, often tend to co-congregate in these areas.

COMPOST FACILITIES AND COMPOST

COMPOST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Facility type – animal manure feedstock	Low	Uncertain	Consistent source of cattle manure as feedstock containing recurring *E. coli* O157:H7 subtype	Moderate as a research objective in model studies; known as risk factor but evidence lacking as to linkage to natural contamination events: specific study would likely require commercial site access and testing to acquire quantitative post-compost process survival and regrowth or contamination and growth; improperly finished and cured compost possible but not likely root cause
Facility type – green waste; no food waste	Low	Uncertain	Contamination of feedstock or finished but not adequately cured compost	As above
Facility type – green waste; industrial oils and food waste	As above			
Facility type – mixed feedstock sources	As above			
Finished to curing intervals	Low	Uncertain	As above	
Facility proximity	Moderate	Uncertain	As above	
Shared farm road traffic	Low	Uncertain	Animal manure feedstock; cattle manure feedstock from a consistent	Moderate as a research objective in model studies; known as risk factor but evidence lacking as to linkage to

COMPOST Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
			contaminated source; contamination of water, production equipment, staged harvest equipment	natural contamination events: specific study would likely require commercial site access and testing to acquire quantitative post-compost process survival and regrowth or contamination and growth; improperly finished and cured compost possible but not likely root cause
Field staging/ spreading grower	High	Likely	Contaminated materials	Moderate as a research objective in model studies; known as risk factor but evidence lacking as to linkage to natural contamination events: specific study would likely require commercial site access and testing to acquire quantitative evidence
Field staging/ spreading adjacent land use	High	Likely	Contaminated materials	As above

WILDLIFE AND INSECTS

WILDLIFE Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Type	High	Likely	Interaction with cattle or contaminated water or feed source	Low as a research objective at this point (known as risk factor) but uncertainty as to linkage to natural contamination events related to outbreaks: specific studies underway but unlikely to acquire quantitative transfer potential evidence
Biology – foraging range	High	Likely	Interaction with recurring source well separated from leafy greens production	High as a research objective at this point (known as risk factor) but uncertainty as to linkage to natural contamination events related to outbreaks: specific studies underway but unlikely to acquire quantitative transfer potential evidence
Interaction with rangelands cattle	As above			
Interaction with cattle holding facilities	As above			
Seasonal behaviors	As above			
Interactions with surface water sources	As above			



Figure 9. Diverse wildlife is recognized as a feature of an open-environment production system. Much of the research directed at this risk factor has characterized birds, the most challenging to control, as a low to moderate source of vector-borne contamination. Shedding of STEC and *E. coli* O157:H7, in general, has been reported to be of low prevalence in the region. Risk exposure has been most associated with flocking birds that interact with cattle/animal feeding operations in close proximity to leafy greens production or long-range foraging behavior. Roosting sites within or adjacent to romaine production is a sensible point of concern.

EVOLUTIONARY GENOMICS of reoccurring *E. coli* O157:H7 (primarily a fundamental knowledge area focus)

GENOMICS Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
SNP diversification rates	This subject area is not specific to root cause analysis although regional cattle are a definitive reservoir and source of the reoccurring outbreak subtype of *E. coli* O157:H7. Mechanisms of transference of STEC and the reoccurring strain is a high priority for root cause analysis and search for solutions to repeated outbreaks. However, clues to an understanding of environmental stability and diversification among this broader genomic clade and sub-clades (highly related isolates found across the CA Central Coast) could lead to more effective pathogen source tracking and novel mitigations. Equally, this knowledge is anticipated to lead to discovery of alternative animal or environmental reservoirs of this foodborne pathogen and pathways to interventions.			High
SNP diversification drivers/stress factors				High
Sub-cluster source tracking				High
Genomic fitness traits – cattle				High
Genomic fitness traits – environmental				High
Genomic fitness traits – vector persistence and shedding				High

BIOAEROSOLS (Inorganic/Organic Dust Particulates)

BIOAEROSOLS Hypothesis Factor	Hypothesis Risk Ranking	Current Risk Rank Probability	Key Co-Factors Modulating Risk Profile	Research Priority Rank
Point source	High	Uncertain	Proximity; persistence in dry manure; particulate fractions with viable STEC; wind speed, wind direction, dispersal vectors; deposition distances	High but the focus is on novel approaches to assess the actual risk exposure and develop predictive models; realistically, low as a research objective at this point (known as risk factor) but uncertainty as to linkage to natural contamination events related to outbreaks: specific study would likely require commercial site access and testing to acquire quantitative evidence
Non-point source	As above			
Wind speed and direction – seasonal prevailing degrees	As above			
Wind speed and direction – seasonal counter-prevailing degrees	As above			
Adjacent source deposition	As above			
Nearby source deposition	As above			
Long-range source deposition	As above			



Figure 9. Bioaerosols, airborne particulates containing organic and inorganic components with living and nonliving biological life forms, from diverse sources, may be transported in the daily winds that are characteristic of the region. Bioaerosol dispersion from a point source or non-point source may be short range or carried over long distances, depending on several factors of particulate size and buoyancy. Aerosol capture devices can indicate relative or quantitative dust and particulates in the airstream over a field, but evidence of deposition and survival of STEC attributed to this risk source has yet to be demonstrated under natural conditions.