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Review

Microgreens—A review of food safety considerations along the farm to fork continuum

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ABSTRACT

The food safety implications of microgreens, an emerging salad crop, have been studied only minimally. The farm to fork continuum of microgreens and sprouts has some overlap in terms of production, physical characteristics, and consumption. This review describes the food safety risk of microgreens as compared to sprouts, potential control points for microgreen production, what is known to date about pathogen transfer in the microgreen production environment, and where microgreens differ from sprouts and their mature vegetable counterparts. The synthesis of published research to date may help to inform Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs) for the emerging microgreen industry.

1. Prevalence of produce-associated foodborne illness

One in ten people worldwide contract illnesses from food contaminated with infectious agents, and 420,000 of those cases result in death (Alegbeleye et al., 2018; Hoffmann et al., 2017). The World Health Organization reported in 2015 that Africa, Southeast Asia, and the Eastern Mediterranean bear the greatest burden, while the Americas and Europe bear the least (World Health Organization, 2015). Nevertheless, the most recent report of confirmed cases of food-borne illness from the Centers for Disease Control and Prevention (CDC) in the United States concluded that in 2015 alone there were 902 food-borne disease outbreaks resulting in 15,202 illnesses, 950 hospitalizations, 15 deaths, and 20 food product recalls (Center for Emerging Diseases, 2015). The true figures could be higher as these events are from confirmed outbreaks. Scallan et al. (2011) reported that an estimated 47.8 million cases of domestically acquired food-borne illness may occur annually in the United States.

A 2013 CDC report on the attribution of illnesses to food commodities showed that 46% of the foods involved in outbreaks are produce, causing 23% of the fatalities (Painter et al., 2013). Further, the CDC's Food-borne Disease Outbreak Surveillance System reported that out of 120 multi-state outbreaks between 2010 and 2014, 17 were from fruits, 15 were from vegetable row crops, 10 were from sprouts, and 9 were from seeded vegetables (e.g. cucumbers, mini peppers) (Crowe et al., 2015). A myriad of pathogens can contaminate produce, including spore-forming bacteria, non-spore forming bacteria, viruses, parasites, and prions. The multi-state outbreak report by Crowe et al. (2015) demonstrates that the most common produce-associated bacterial pathogens are *Salmonella enterica*, *Listeria monocytogenes*, and shiga toxin-producing *Escherichia coli*. Human norovirus, the leading cause of food-associated acute gastroenteritis, is responsible for 5% of all food-borne illnesses of known etiology in the United States (Scallan et al., 2011) and 65% of those in Canada (Thomas et al., 2013). A search on September 7, 2018 for 'norovirus' and 'food' in the CDC's National Outbreak Reporting System (NORS) Database revealed that norovirus is the major cause of outbreaks associated with leafy greens. After multiple ingredient foods and foods considered 'unclassifiable,' 'vegetable row crops,' 'other,' 'mollusks,' and 'fruits' are the most common food categories implicated in norovirus outbreaks.

A 2013 report by the European Food Safety Authority (EFSA) attributed an increase in cases of foodborne illness (from 18% to 26%), hospitalizations (from 8% to 35%) and deaths (5% to 46%) between 2007 and 2011 to one large verocytotoxigenic *Escherichia coli* (VTEC) outbreak in Germany in 2011. Fenugreek sprouts were identified as the infected food and over 3800 people were affected (European Food Safety Authority, 2013). The EFSA later reported that active surveillance of eight European Union (EU) member states revealed one sample of 344 collected was positive in 2016 compared to zero positive samples out of 444 collected from six member states in 2013 (European Food Safety Authority, 2017). Produce-associated outbreaks in the United States have also increased in the last two decades, from 8% of food-borne illness outbreaks between 1998 and 2001 to 16% between 2010

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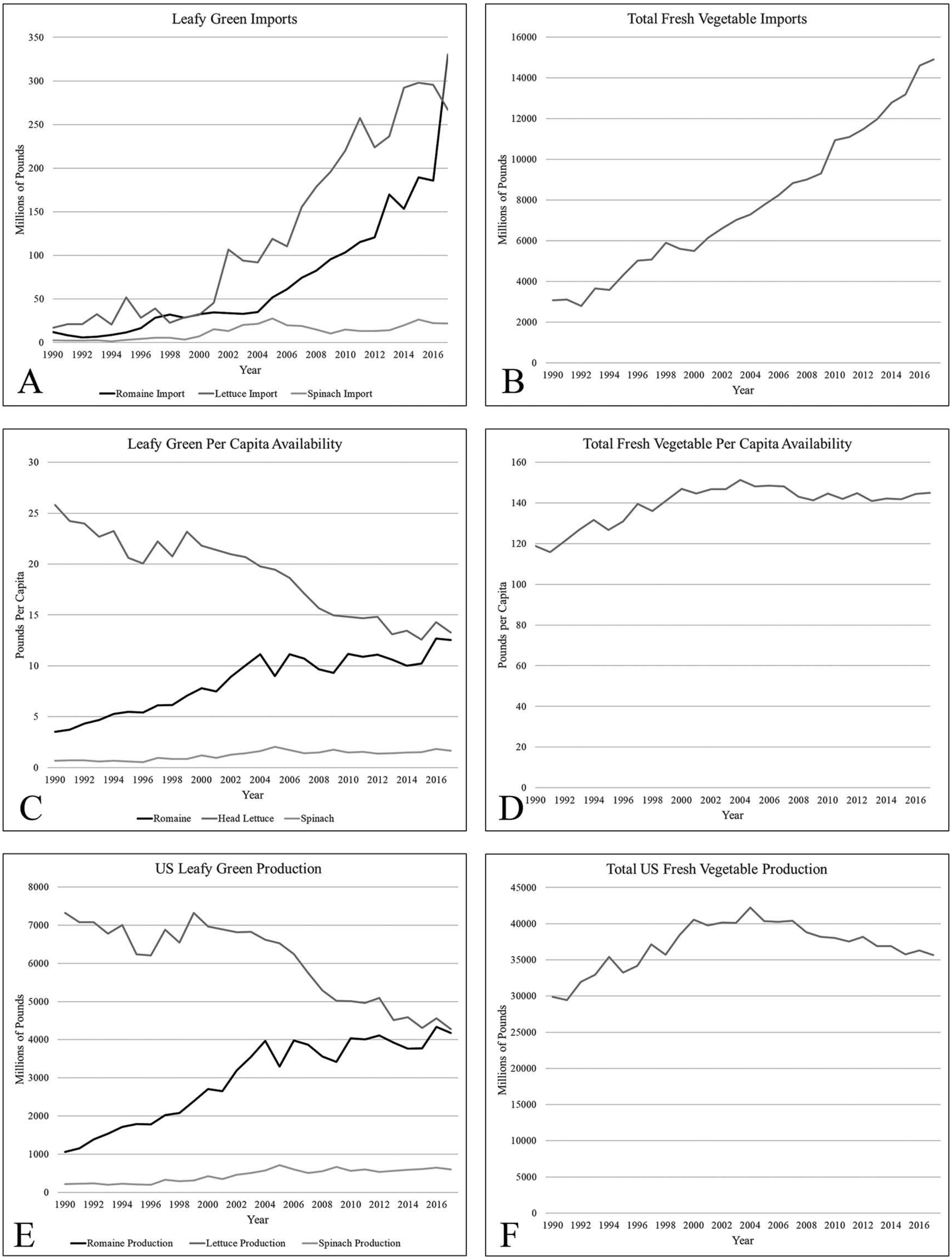

Fig. 1. Leafy green consumption and availability.
Lettuce, leafy green, and total fresh vegetable imports (A and B), per capita availability (C and D), and production (E and F) in the United States from 1990 to 2016. Source: ERS/USDA, Accessed June 4, 2018.

and 2013 (Bennett et al., 2018).

Alegbeleye et al. (2018) postulated that increases in produce-related outbreaks are at least partially due to improved surveillance and reporting. However, they suggest a true increase in produce-associated illness may simply be a result of increased consumption of fruits and vegetables. Data collected by the United States Department of Agriculture's Economic Research Service (ERS/USDA) from 1990 to 2016 show that while head lettuce availability per capita and domestic production has gone down, there has been an increase in availability and production of romaine lettuce and a slight increase in spinach availability. There has also been an increase in imported fresh vegetables that is suggested to correspond with an increase in imported Romaine and head lettuce (Fig. 1).

An increase in importing supports the assertion by Alegbeleye et al. (2018) that agriculture has become more globalized. Globalization adds challenges in regulating food safety since practices differ between countries, such as water quality management and waste water treatment. According to a report by the International Food Policy Research

Institute (IFPRI), developing countries often have difficulty meeting the strict food safety requirements of developed nations (Käferstein, 2003). Lastly, agriculture has become more intensive due to increased demand for fresh fruits and vegetables, so produce may be more likely to be in close proximity to potential sources of contamination such as livestock. In these settings, fresh produce may become contaminated via the soil, irrigation water, wildlife, insects, livestock, pets, or soil amendments such as manure (Alegbeleye et al., 2018).

As the consumption of fresh produce is changing, so are the types of fresh produce available. Microgreens, which are the immature shoots of products such as sunflower, peas, chard, beets, spinach, kale, and cilantro, are an emerging salad crop. They are often grown in trays indoors or in greenhouses and are touted for their reported high nutrient content. Microgreens have recently grown in popularity in developed countries due to increased interest in gourmet cooking, healthy eating, and indoor gardening. They have a relatively short shelf life even in refrigeration and are used in small quantities as garnishes, toppings, or seasonings (Delian et al., 2015; Kyriacou et al., 2016; Mir et al., 2017; Treadwell et al., 2010; Xiao et al., 2012).

Microgreens may be easily confused with sprouted seeds, which have been frequently implicated in food-borne illness (Gensheimer and Gubernot, 2016). However, while microgreens share some characteristics with sprouts, they share others with fresh herbs and petite greens. Examples of fresh herbs include basil, thyme, and cilantro and examples of petite greens include baby spinach and spring mix. While there is a growing body of literature on both microgreen nutrition and physiology, only eight studies since 2009 have specifically examined the food safety risk of microgreens. However, leafy green and sprout safety has been studied extensively. The purpose of this review is to compare microgreens to other raw salad crops previously shown to be linked to food-borne illness and identify potential control points given what is currently known about how raw produce is colonized by disease-causing microorganisms.

2. Traits of high-risk crops: how microgreens compare

Produce can become contaminated at any point along the farm to fork continuum. Common control points for growers include irrigation water, soil amendments such as manure or compost, livestock and wild animal fecal contamination, worker health and hygiene, field and harvest sanitation, sanitation of packing facilities, post-harvest water and handling, value-added processing, storage, transportation, and distribution (Olaimat and Holley, 2012; Suslow, 2003). The crops with the greatest risk of becoming contaminated with human pathogens include lettuce, spinach, parsley, basil, berries, green onions, melons, sprouts, and tomatoes (Alegbeleye et al., 2018). Each of these crops have earned their high-risk status because of growing conditions that facilitate the growth or transfer of microorganisms, production methods that expose the product to contaminants from animals or humans, and physiological characteristics of the plant that facilitate contact and binding with microorganisms. Microgreens share some traits with these high-risk crops.

2.1. Tissue damage increases susceptibility

Harvesting by cutting may increase susceptibility to contamination. For example, tomato stem scars result from picking or cutting a tomato from its stem during harvest, and research in this area demonstrates that tissue damage can expose produce to contaminants. Lin and Wei (1997) demonstrated that *Salmonella* Montevideo clusters around tomato stem scars at 10^3 colony forming units (CFU). At higher inoculum doses of 10^4 and 10^5 CFU, *Salmonella* Montevideo spread to the interior of the tomato. Lettuce and spinach are often vehicles of produce-associated food-borne illness (Gao et al., 2016; Waitt et al., 2014; Wang et al., 2017). Damage to leaves, stems, and roots sustained during post-harvest processing may facilitate pathogen contamination. Like tomatoes, lettuce is harvested by cutting, and the cut site may be a route of entry for pathogens. Aruscavage et al. (2008) demonstrated that *Escherichia coli* O157:H7 survived better on lettuce split along the central vein compared to healthy, undamaged leaves. Microgreen harvesting also involves cutting by hand just above the soil line, but to our knowledge there is no research indicating whether the cut end of microgreens are susceptible to contamination as observed in lettuce and tomatoes. Sprouted seed production, however, has no cutting step (United States and Food Drug Administration, 2017a). Therefore, contamination at the cut edge is one contamination susceptibility of microgreens not shared by sprouted seeds.

Surface characteristics combined with tissue damage of lettuce leaves and other leafy greens may create opportunities for contamination. For example, Wang et al. (2017) and Gao et al. (2016) have demonstrated that lettuce leaf surfaces express glycoproteins that are biochemically similar to histo blood group antigens (HBGA) in mammals and serve as attachment sites for norovirus capsid proteins. Human noroviruses are the primary cause of foodborne illness associated with leafy greens (Sivapalasingam et al., 2004; Herman et al., 2015; Bennett et al., 2018). Gao et al. (2016) demonstrated that enzymatic degradation of red leaf lettuce, Romaine lettuce, and celery tissue by cellulase R10 increases binding of human norovirus capsid proteins, likely due to exposing additional binding sites. However, binding of norovirus capsid protein to HBGAs did not occur with basil, indicating that pathogen attachment may depend at least partially on plant variety.

Lectins and adhesins on leaf surfaces also act as binding sites for bacteria such as *Salmonella* and *E. coli* O157:H7. These pathogens are implicated in many of the outbreaks traced to spinach and lettuce (Deng and Gibson, 2017). A review by Berger et al. (2010) concluded that plant variety and bacterial species both play a role in the ability of contaminants to attach to plant surfaces. Even among *Salmonella enterica* serovars, they found that there is considerable variation in attachment ability and mechanism. Major cell components involved in attachment include the pilus curli, the O antigen capsule, and cellulose synthesis necessary for biofilm formation. *E. coli* variants also use curli when attaching to tomatoes, spinach, and alfalfa roots. *E. coli* attachment to leafy vegetables is also aided by its filamentous type III secretion system and its flagellum (Berger et al., 2010; Olaimat and Holley, 2012). Such a phenomenon demonstrated on the leaves of full sized vegetables suggest that it is likely to occur on microgreen leaves as well, though more studies are needed to determine the susceptibility of individual microgreen varieties to particular pathogens.

2.2. Hand-harvesting and farm worker hygiene

Because microgreens are typically harvested by hand, it is worth considering the risks that producers themselves contribute through inadequate hygiene. *Salmonella* is the most common cause of produce-borne infections, so an extensive body of research has been focused on understanding how this animal fecal organism finds its way to fresh fruits and vegetables (Olaimat and Holley, 2012; Waitt et al., 2014). Inadequate worker hygiene is a major contributing factor to contamination of produce by human pathogens, especially for hand-harvested crops like strawberries (Moore et al., 2015). Of the pathogens identified in a review by Todd et al. (2009) of outbreaks involving food workers between 1927 and 2006, *Salmonella* species and norovirus were the most prevalent for the bacterial and viral categories, respectively, for all food vehicles studied. Specifically, in produce, however, *Salmonella* was only implicated in 4.6% of outbreaks and *Shigella* was the most commonly implicated pathogen, representing 21.2% of outbreaks involving food handlers. Todd et al. (2009) focused primarily on the service end of the food continuum, particularly restaurant workers, who made up the majority of reports.

Inadequate hygiene practices by farm workers also pose a risk at the production end of the food continuum. Bartz et al. (2017) conducted a matched-pair epidemiological study of 11 farms and calculated the odds ratios of the presence of indicator organisms on worker hands to the presence of indicator organisms on produce. The indicator organisms chosen were total coliforms, *E. coli*, *Enterococcus*, and coliphage and the target produce included cantaloupe, jalapeno peppers, and tomatoes. When *E. coli* was found on hands, the handled produce was nine times more likely to contain *E. coli*. When coliphage was present on worker hands, the handled produce was eight times more likely to contain coliphage. Surprisingly, there was no significant relationship between bacteria or phage in either soil or irrigation water. These data suggest that transfer from worker hands was the main contributor of contaminants.

When the production environment and harvesting techniques are combined with specific physiological interactions between produce and pathogens, the risk is compounded. Sprouts, the agricultural product most closely resembling microgreens, will be described shortly as a perfect storm of these three factors. Microgreens are similar to high-risk crops such as lettuce, berries, green onions, melons, sprouts, and tomatoes because they, too, are frequently consumed raw. Good Agricultural Practices (GAP) and Good Handling Practices (GHP) with respect to personal hygiene and glove use are therefore even more crucial to prevent microgreens from suffering the same fate as other uncooked produce.

2.3. Sprouts: an ideal disease vector

Sprouted seeds are an agricultural product most closely resembling microgreens. These young germinated seeds are often eaten raw (U.S. Department of Health and Human Services, 2015) and exemplify the intersection of production, growth, and handling conditions that allow pathogens to thrive. A search for "sprouts" in the CDC's Food Outbreak Online Database (FOOD) showed that products such as alfalfa, clover, and bean sprouts have been implicated in 53 outbreaks, 1876 illnesses, 209 hospitalizations, and numerous product recalls between 1998 and 2016 (Table 1). *Salmonella enterica*, shiga-toxin producing *E. coli*, *L. monocytogenes*, and human norovirus genogroup I were implicated in the 1876 food-borne illnesses from sprouts between 1998 and 2016, with *Salmonella enterica* alone responsible for 1675 illnesses (Table 2). The illnesses associated with norovirus genogroup I were from a single outbreak. In early 2018, the sandwich franchise Jimmy John's recalled alfalfa sprouts from its 2727 locations due to patrons in Wisconsin, Minnesota, and Illinois becoming ill with *Salmonella* serovar Montevideo that could be traced back to two seed lots from two Minnesota growers (Flynn, 2018).

Interestingly, Salmonella enterica appears to be the cause of more than three quarters of the reported illnesses resulting from contaminated sprouts (Table 1), and organic soil amendments may be a contributing factor (Jung et al., 2014). In particular, alfalfa sprouts appear to have been the most common variety among reported sprout-linked illnesses between 1998 and 2016, followed by mung bean and clover sprouts. One outbreak (32 illnesses) was traced specifically to alfalfa seeds (Table 3). Alfalfa and clover seeds are produced in large fields primarily for animal forage, and may be fertilized with manure. A subset of these seeds are sold to sprout producers. If proper sterilization or heat-pelleting of manure is not performed prior to application, seeds used for sprouts may be contaminated (Taormina et al., 1999).

Table 1
Sprout outbreaks by year. Morbidity and mortality related to foodborne disease outbreaks linked to consumption of sprouts in the U.S. from 1998 to 2016.
Source: Centers for Disease Control and Prevention National Outbreak Reporting System (NORS), Accessed June 4, 2018.

Year	Outbreaks	Illnesses	Hospitalizations	Deaths
1998–2001	12	711	56	0
2002–2005	10	166	16	1
2006–2009	11	425	31	0
2010–2013	11	293	49	1
2014–2017	9	272	57	3
Total	53	1867	209	5

Table 2
Sprout outbreaks by etiology. Sprout outbreaks by etiology from 1998 to 2016. Source: Centers for Disease Control and Prevention National Outbreak Reporting System (NORS). Accessed June 4, 2018.

Etiology	Illnesses	Hospitalizations	Deaths
L. monocytogenes	27	21	2
Norovirus genogroup I	32	0	0
S. enterica	1675	160	2
Shiga-toxin producing *E. coli*	133	28	1
Total	1867	209	5

Table 3
Sprout illnesses by food vehicle. Sprout illnesses by food vehicle from 1998 to 2016.
Source: Centers for Disease Control and Prevention National Outbreak Reporting System (NORS). Accessed June 4, 2018.

Product	Total illnesses
Alfalfa seeds	32
Alfalfa sprouts	1059
Bean sprouts	68
Clover sprouts	212
Mung bean sprouts	394
Sprouts, unspecified	55

Sprouts are produced by soaking seeds and then germinating them in a moist environment for approximately 5–7 days. Therefore, they may be exposed to temperatures and moisture levels optimal for the growth of mesophilic bacteria, including many human pathogens. Germination conditions provide ample time for pathogen proliferation and internalization (Warriner et al., 2005). Multiple studies have shown that pathogenic bacteria are capable of proliferating in the sprout germination environment, including enterohemorrhagic *E. coli* on radish sprouts (Itoh et al., 1998) and *Vibrio cholerae* O1, *Salmonella typhi*, and *Escherichia coli* O157:H7 in alfalfa sprouts (Castro-Rosas and Escartin, 2000). Furthermore, there is evidence that growth of *Salmonella* during the sprouting process is capable of leading to outbreaks (Erdozain et al., 2013; Stewart et al., 2001).

By contrast, microgreens are immature seedlings of edible plants wherein their seeds are soaked only briefly, if at all, and harvested above the growth media after 10 to 21 days, between the opening of the cotyledon and the showing of the first set of true leaves (Fig. 2). Both microgreens and sprouts are often grown in greenhouses, high tunnels, and climate-controlled buildings. Since sprouted seeds have been implicated in a large number of high profile food-borne illness outbreaks as well as recalls over the past two decades (Gensheimer and Gubernot, 2016), this has led to the suspicion that microgreens may be similarly susceptible. Indeed, there are enough similarities between microgreens and sprouts to warrant thorough investigation into this emerging product. So far, there are no reported outbreaks or illnesses associated with microgreens. However, there have been six microgreen product recalls since 2016 due to contamination by either *Salmonella* or *L. monocytogenes* in the finished product as reported by the FDA Food Recalls, Withdrawals, and Safety Alerts Database (US Food and Drug Administration, 2016, 2017b, 2018) and by the Canadian equivalent (Canadian Food Inspection Agency, 2018a, 2018b, 2018c). No consumer illnesses were reported; in all cases the contamination was discovered during routine quality control procedures.

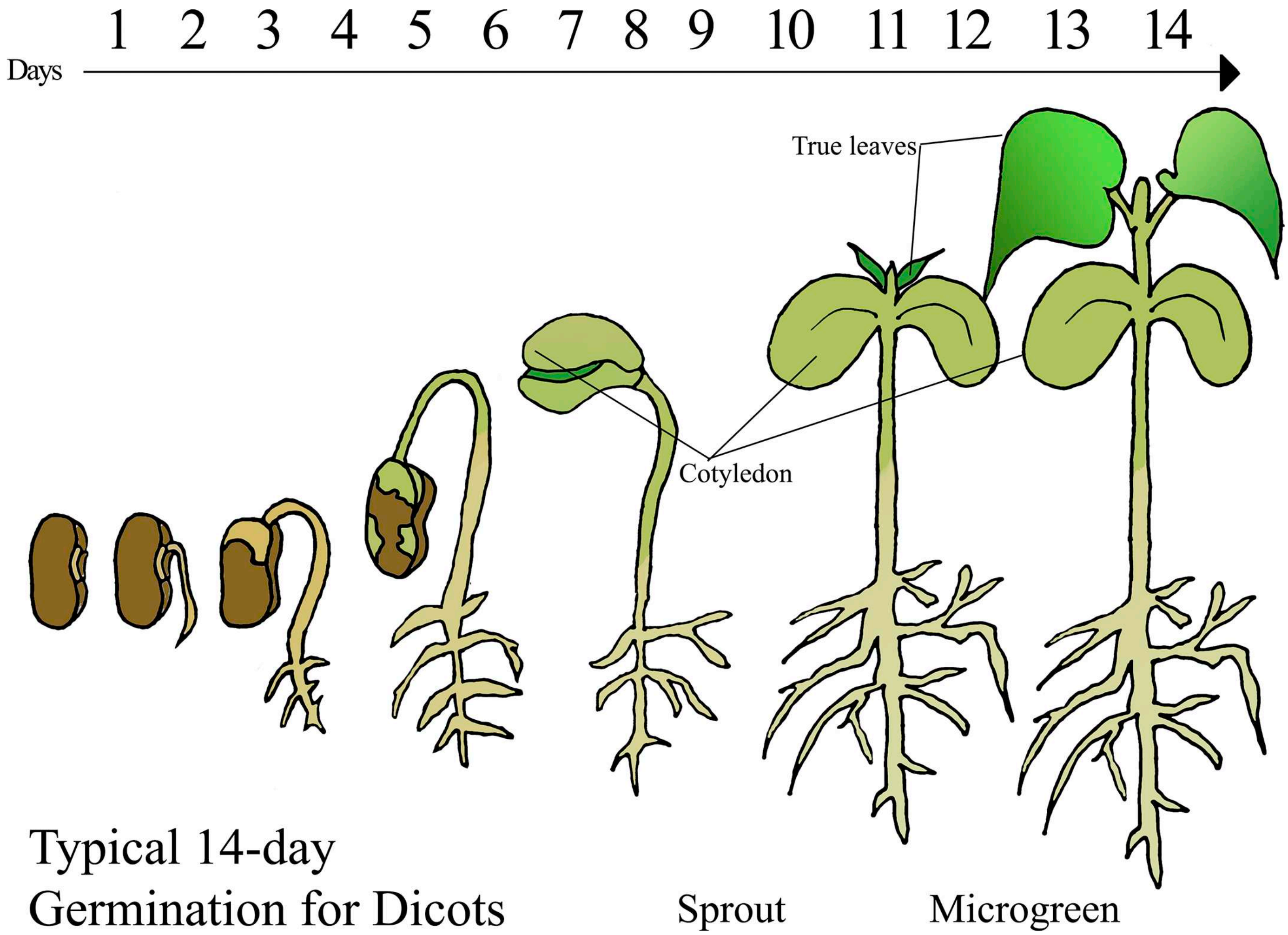

Fig. 2. Microgreens and sprouts differ by age at harvest.
A typical 14-day germination period for a dicot, using the common garden bean as an example. Germination period for microgreens and sprouts varies by plant variety.

3. The Produce Safety Rule and guidance for the sprout industry

The Food Safety Modernization Act (FSMA) was signed into law on 2011 as a sweeping measure to prevent food contamination. The Produce Safety Rule (81 FR 57784) is the section of the FSMA finalized in November 2015 (U.S. Department of Health and Human Services, 2015) that focuses on the prevention of contamination before, during, and after the production of fresh fruits and vegetables typically eaten raw. The Produce Safety Rule contains specific guidelines for sprouts, but not for microgreens. Requirements for sprouts include routine testing of the growing environment and agricultural water for the presence of *Listeria* species, testing each batch of spent sprout irrigation water or sprouts for *E. coli* O157:H7, *Salmonella* species, and other pathogens when necessary. The rule also requires that proper corrective actions are taken if contamination is found.

Responses to comments on the Produce Safety Rule (Comments-Subpart A pg. 74497) clarify that microgreens, fresh herbs, and edible flowers are all covered under the Produce Safety Rule Part 112 “Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption” that governs all other produce eaten raw. This is because, despite microgreens' similarities to sprouts, the FDA maintains that microgreens are not sprouts due to their age at harvest and differences in harvesting practices and are therefore not covered under the sprout requirements in Part 112 Sub-part M of the rule. However, the FDA encourages producers of microgreens to voluntarily comply with the sprout guidelines. For microgreen operations that utilize hydroponics and aquaponics, the FDA recommends that producers comply with the agricultural water and soil amendment provisions addressed in Part 112 sub-part E and F, respectively.

3.1. Good Agricultural Practices

Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs) are voluntary audits of on-farm food safety practices that produce growers may undergo in order to demonstrate compliance with the standards set forth by produce industry guidance documents. Commodity specific guidelines include the 1998 “Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (Fda1998),” the updated 2011 “Produce GAPs Harmonized Food Safety Standard (United States Department of Agriculture, 2018),” and “Compliance with and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations: Guidance for Industry,” (United States Food and Drug Administration, 2017a, 2017b). These are non-binding recommendations that assist growers in complying with the Produce Safety Rule. The Produce Safety Alliance (PSA) and the Sprout Safety Alliance (SSA) exist to help growers comply with the requirements of the Produce Safety Rule by offering training, educational programming, and assistance with GAP self-audits (Calvin, 2013).

3.2. Are commodity specific guidelines for microgreens needed?

There are presently no commodity specific guidelines for microgreens. It may not be necessary to establish a separate sub-part to the Produce Safety Rule specifically for microgreens, as many of the general guidelines are sufficient to address any potential issues related to microgreens. However, because microgreens share some traits in common with full-sized fresh produce and other traits in common with sprouts, it may be necessary to develop a guidance for industry to help microgreens growers navigate and comply with the various sub-parts of Part 112 of the Produce Safety Rule that apply to them.

4. Potential control points for microgreens

Microgreens have the potential to become contaminated by pathogens from seed to harvest. Possible control points on the production continuum are outlined here. Some of these control points are common to all raw produce, while some are unique to microgreens.

4.1. Irrigation water and irrigation methods

Microgreens are often grown in greenhouses, high tunnels, and climate-controlled buildings where contact with livestock, insects, and wildlife is minimal. Additionally, indoor and greenhouse operations tend not to use fertilizers, manure or otherwise, because the product is harvested after only one to three weeks (Treadwell et al., 2010; Xiao, 2013; Xiao et al., 2014b). Irrigation water, however, is of particular concern when it comes to sprouts and microgreens, especially those grown hydroponically. Studies conducted in the field indicated that norovirus, for example, can directly contact and attach to vegetables and fruits from experimentally contaminated irrigation water (Alum et al., 2011; Stine et al., 2005).

The type of irrigation technique affects the risk of contamination. Produce irrigation water acquires pathogens during transportation through either canals, ditches, or pipes. Outdoor transportation exposes water to soil bacteria and parasites while pipes expose the water supply to biofilms. Some types of "sustainable" irrigation systems may compound the risk of microbial contamination, such as gray-water recycling and rainwater collection tanks. Drip irrigation reduces the risk of produce contamination compared to overhead spray irrigation due to limiting exposure of the edible portion of the plants to the water (Painter et al., 2013; Solomon et al., 2002).

Surface water sources such as nearby rivers, lakes, and streams have been to blame for many large outbreaks of food-borne illness. In 2011, 390 elementary schools and child care facilities contracted norovirus from contaminated frozen strawberries imported from China. The investigators hypothesized that, due to the size of the outbreak, the source may have been norovirus-contaminated irrigation water (Bernard et al., 2014). A 2012 outbreak of *Salmonella* Litchfield in Australia affecting 26 people was traced back to contaminated river water that was being used to wash papayas. In the United States, an *E. coli* O157:H7 outbreak in 2006 in prepackaged spinach affecting 205 people was traced back to contaminated surface water; the clinical isolate was detected in nearby river water and in cow and pig feces from a nearby farm (Gelting, 2007). Four outbreaks of acute gastroenteritis associated with norovirus isolates from cabbage kimchi occurred in South Korea between 2008 and 2012 and were traced back to contaminated irrigation water (Cho et al., 2014).

Since microgreens are grown in trays in greenhouses or on artificially lit shelves indoors, producers may be more likely to water from municipal sources, groundwater, gray water, or collected rainwater. A review by Uyttendaele et al. (2015) concluded that municipal water is of the best microbial quality, followed by groundwater, gray water, and collected rainwater. Groundwater quality can be compromised, however, if the reservoir is too shallow, if heavy rainfall floods reservoirs with feces and microorganisms on land, or a nearby septic system or sewage line leaks. Roof-top collected rainwater may become contaminated by bird droppings and insects found on rooftops.

4.2. Decontamination of the seed

Seed contamination is a well-known problem in the sprout industry. If seeds are contaminated, pathogens can become internalized from the beginning of the growing process and once incorporated are very difficult to remove (Wang and Kniel, 2016). Because of this, a significant body of literature has grown out of efforts to determine effective seed disinfection procedures. The FDA cites 20,000 ppm calcium hypochlorite as the standard method of chemical disinfection (US Food and Drug Administration, 1999), though adoption of this practice by growers may vary widely. Harrison (2017) reported, for example, that many growers selling at farmers' markets had limited food safety knowledge related to fresh produce, lending to the assumption that disinfection practices are not standard. Additionally, sprout producers who are seeking organic certification may not be permitted to use chlorine compounds on their products at levels exceeding the Environmental Protection Agency's standards for drinking water, which are 0.8 ppm (Organic Standards, EPA Water Standards).

A review of sprout seed disinfection techniques by Ding et al. (2013) found that across 44 published articles, 18 of which tested the FDA recommendation of 20,000 ppm calcium hypochlorite, the standard 10 to 15-minute soak enabled a mean reduction in bacterial load of 3.08 log CFU/g with a standard deviation of 2.03 log CFU/g. The concentrated hypochlorite treatment had roughly twice the variability of the non-chemical methods such as heat treatment and irradiation compared in Ding et al. (2013), likely due to slightly differing protocols used by growers and the physical characteristics of the seed. For example, rough textured or scarified seeds were more difficult to disinfect than smooth seeds. It was hypothesized that bacteria and viruses are able to hide in the crevices of the seed surface and evade contact with disinfectants. Microgreen varieties such as pea shoots and sunflower are smooth in texture, but other varieties such as chard and beet have a rough, irregular surface (Fig. 3). Therefore, investigations into seed disinfection strategies for different microgreen varieties may be necessary.

With sprouts and microgreens, germination rate is a critical factor in production. Ding et al. (2013) demonstrated that physical methods such as heat treatment also boast high log CFU/g reductions, but it is a balancing act to achieve adequate reduction without compromising germination rate. High pressure treatment, out of all of the methods surveyed by Ding et al. (2013), demonstrated the lowest variability (standard deviation = 0.94 log CFU/g) and the highest mean reduction of 5.09 log CFU/g with insignificant effects on seed germination rate. High-pressure treatment also has the advantage of being amenable to organic certification, though potentially more expensive for small operations than chemical treatments because it requires special equipment (Wuytack et al., 2003).

Biological control is a relatively new attempt at dealing with seed contamination, though it is difficult to assess effectiveness of the methods because of the very specific environmental conditions of each approach. Studies have involved competition by communities of normal flora (Matos and Garland, 2005) and bacteriophage (Kocharunchitt et al., 2009) to control levels of unwanted bacteria with some success. There are potential health risks associated with these methods due to the many unknowns involved, and may be difficult to scale beyond the bench.

4.3. The relationship between post-harvest washing, spoilage, and contamination

Since microgreens have a relatively short shelf life of three to five days even in refrigeration and are used in small quantities (Kou et al., 2014), it is important to determine if there is any connection between

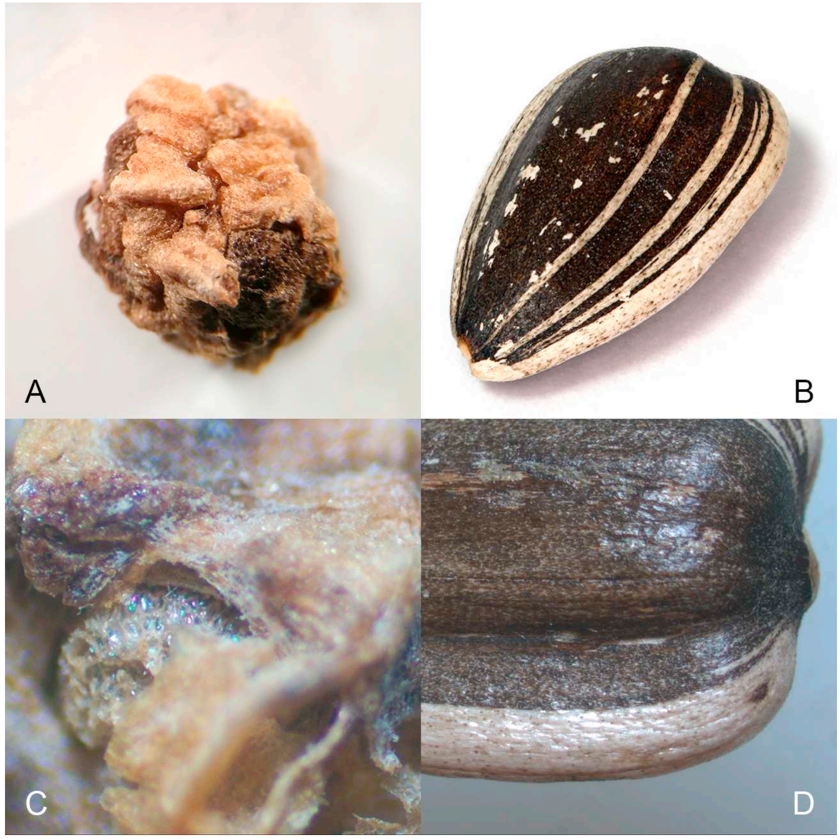

Fig. 3. Differences in seed topography.
A) Swiss chard seed 17.5×, Olympus SZ60; B) sunflower seed, public domain; C) Swiss chard seed 150×, AccuScope 3072/Excelis SMZ143; D) sunflower seed 150×, AccuScope 3072/Excelis SMZ143.

produce spoilage and contamination by human pathogens. As stated in a previous section, plant tissue damage creates opportunities for pathogen attachment or entry. In addition to damage by human handlers and harvesting tools (Lin and Wei, 1997; Moore et al., 2015; Bartz et al., 2017), enzymatic digestion by spoilage microorganisms may facilitate contamination. Gao et al. (2016) demonstrated this possibility in their study on virus attachment to lettuce leaves. Virus attachment to the leaf surface increased significantly after enzymatic digestion by cellulase. They also found that virus attachment increased when the leaf cuticle was peeled back, suggesting that the cuticle offers some protection.

Damage may also occur during post-harvest washing. In an effort to determine if post-harvest calcium chloride wash would have a measurable effect on shelf life of broccoli microgreens, Kou et al. (2015) found that the washing procedure itself decreased shelf life from 21 days to 14 days due to mechanical damage during rinsing, spinning, and drying. They also found that chlorine washes at 50 and 100 ppm were not effective at altering shelf life.

Refrigeration temperatures may also play a role. Kou et al. (2013) found that buckwheat microgreens stored at 1 °C suffered tissue damage, whereas buckwheat microgreens stored at 5 °C and 10 °C did not. The tissue damage corresponded to a greater increase in APC toward the end of the storage period. However, Xiao et al. (2014b) found that radish microgreens retained their quality best at 1 °C compared to 5 °C and 10 °C. It is possible that there is a differential tolerance to temperature among microgreen varieties. They also found that a 100 ppm chlorine wash did not extend shelf life as aerobic mesophilic bacteria (AMB) increased by almost 4 log CFU by the seventh day of storage.

It appears to be important to prevent the growth of both pathogenic and spoilage related microorganisms earlier in the production chain, especially since post-harvest washing may cause tissue damage. Kou et al. (2014) tested the effects of a pre-harvest spray of calcium chloride, rather than a post-harvest wash. The spray seemed to have a beneficial effect on the post-harvest quality and shelf life of broccoli microgreens based on reduced tissue electrolyte leakage and lower microbial growth during storage.

5. Microgreen safety

While there is a growing body of work on the health benefits of microgreens, there are very few reports on microgreen safety. Only eight reports of specific investigations into food safety risk of microgreens have been published to date, the first of which was Lee et al. (2009). After washing Chinese cabbage *Brassica campestris* var. *narinosa* microgreens in distilled water and two different concentrations of chlorine (50 ppm and 100 ppm) at two different water temperatures (5 °C and 25 °C), post-storage quality measurements and APC were compared. The data suggest that both concentrations of chlorinated water reduced APC more effectively than non-chlorinated water. Warmer wash water appeared to have a slightly stronger effect on reducing APC compared to cooler wash water. However, by the sixth day of storage, APC had increased from 7 log CFU to greater than 9 log CFU for test groups and controls. Additionally, the authors stated that as other measures of microgreen quality decreased, APC increased.

Chandra et al. (2012) studied microgreens of Chinese cabbage (*Brassica campestris* var. *narinosa*) and compared quality measurements, total coliforms, and APC after washing in four disinfectant mixtures and holding at 5 °C for 9 days. The disinfectant mixtures used were tap water (control), 100 mL/L chlorine, a citric acid/ascorbic acid mixture (0.25 percent w/v of each), and a 0.50 percent w/v citric acid solution followed by a 50 percent ethanol spray. The effect of packaging material was also considered. Two sets of microgreens were treated by the aforementioned methods and then were stored in either polypropylene or polyethylene containers. In both container types, APC was lower in microgreens treated with 100 ppm chlorine and the citric acid/ethanol treatment. Similar to Lee et al. (2009), counts rebounded around the sixth day to a log CFU level exceeding pre-wash levels.

Total coliform counts demonstrated by Chandra et al. (2012) sharply increased over three days in storage, and then began to slightly decrease after the 9th day. They failed to return to baseline levels. This pattern was observed regardless of treatment method or storage container, although the 100 ppm chlorine and citric acid/ethanol spray treatments resulted in overall lower log CFU/g of coliform bacteria compared to the other treatments for both types of packaging. These results were reported to be statistically significant at a p-value less than 0.05. The researchers stated that the reason for this decrease in proliferation is unclear and may be a result of multiple confounding variables in the storage environment including water content, pH, storage temperature, and relative humidity. Nevertheless, it can be surmised by these results that none of the sanitizing treatments tested were able to effectively reduce the log CFU/g of coliform bacteria on cabbage microgreens sufficiently enough to prevent regrowth.

Xiao et al. (2014) performed several experiments exploring the proliferation of two strains of *E. coli* on experimentally contaminated radish seeds. The starting inoculation levels were compared to the harvest levels of these *E. coli* strains at both the sprout stage and the microgreen stage. The microgreen stage had consistently lower counts at harvest relative to the inoculation level, even though the microgreens and sprouts came from the same batch of contaminated seeds. Watering overhead or from below made no significant difference in the proliferation of *E. coli* on the edible parts of the microgreen; however, the inedible parts showed higher growth that appeared to correspond with higher levels in the soil.

Xiao et al. (2015) compared the type of growth media on the proliferation of *E. coli* O157:H7 from seed to harvest of radish microgreens. Radish seeds were inoculated at low and high levels of *E. coli* and radish microgreens were grown in a peat moss based soil substitute and in a hydroponic system. Compared to soil-grown microgreens, there was a large, statistically significant increase in proliferation of *E. coli* on the hydroponically grown plants. This occurred on both the edible and inedible plant parts as well as the hydroponic water. The researchers suggested that there could be competitive microflora in the germination mix that inhibits the growth of *E. coli* compared to the hydroponic media.

These findings suggest that exposure to moisture is a significant contributing factor to the spread of *E. coli* in microgreen growing systems. In addition to *E. coli* cell counts, the researchers also assessed the spatial distribution of *E. coli* cells on various parts of the microgreen using a green fluorescent protein (GFP) labeled *E. coli* strain viewed with laser confocal scanning microscopy. Spatial analysis showed that the seed coat was the most densely populated part of the microgreen, whereas the hypocotyl and cotyledon were much less densely populated.

A comparison of the native microbial populations on different types of growth media was performed by Di Gioia et al. (2017). They measured AMB, yeast and molds (YM), Enterobacteriaceae, and *E. coli*. Their data showed that food-grade plastic mats had the lowest overall AMB and YM levels, whereas peat had the highest levels. Peat and jute-kenaf grown microgreens had the highest levels of AMB and YM, and peat had the highest levels of Enterobacteriaceae. The microgreens grown on textile fibers and food-grade plastic mats had no detectable levels of Enterobacteriaceae or *E. coli* in the edible portion of the plant, indicating that they were not as easily transferred to the edible part of the plant from those media. Conversely, the jute-kenaf fiber growing media did not have detectable levels of Enterobacteriaceae but this group of bacteria was strongly detected on the microgreens.

Researchers have also investigated the role of contaminated hydroponic nutrient water on the persistence and transmission of viruses, using murine norovirus (MNV) as a surrogate for human norovirus, the primary cause of food-borne disease outbreaks in the US. Wang and Kniel (2016) grew kale and mustard microgreens in a hydroponic system that was artificially contaminated by 3.5 log PFU/mL of MNV on the 8th day of growth. Water and microgreen tissue samples were collected at 2, 4, 8, and 12 h immediately following inoculation. After day 8, water and microgreen tissue samples were collected daily until the 12th day. This design enabled monitoring of detectable levels of virus taken up by the plants in addition to the rate of die-off toward the end of harvesting.

Virus survival immediately following inoculation remained relatively consistent at ~2 log plaque-forming units per milliliter of water (PFU/mL) for up to 12 h of sampling. By day 12, MNV only decreased by around 1 log PFU/sample (statistically significant) in both varieties of microgreens. This decrease was similar for internalized virus as well as its concentration in the hydroponic nutrient water. The virus was also detected at around 1–2 log PFU/mL in the hydroponic water for up to 16 days post-inoculation and contaminated the next crop of microgreens at detectable levels in both root and shoot tissue. These findings demonstrate that MNV can persist at detectable levels in hydroponic systems for at least several weeks from an initial inoculation of 3.5 log PFU/mL. There were no statistically significant differences overall between kale and mustard.

Wright and Holden (2018) studied the colonization of nine varieties of microgreens by shiga-toxin producing *E. coli* serovar Sakai (STEC). Experiments were conducted on seeds contaminated directly at 3 log CFU/g and on seeds grown with contaminated irrigation water at 7 log CFU/g. Varieties tested were amaranth, broccoli, kale, mustard, coriander, rocket, basil, parsley, and radish. Colonization for eight of the nine microgreen varieties exceeded 8 log CFU/g of fresh weight. Basil was the only variety to show a final STEC level of less than 8 log CFU/g with 7.21 log CFU/g of fresh weight. Previous research by Gao et al. (2016) has shown that basil is also less likely to be colonized by a norovirus surrogate, again pointing to possible plant variety differences.

Reed et al. (2018) was able to demonstrate differences in colonization between *Salmonella enterica* serovars Hartford and Cubana on alfalfa sprouts and Swiss chard microgreens. External factors tested were growth media, storage time, contamination of either seed or water, and inoculation level. For sprouts and microgreens grown from contaminated seeds, increasing the inoculation level from 10 to 100 CFU/g of seed had the most influence on colonization of both microgreens and sprouts, regardless of serovar. However, for sprouts, increasing storage time from 7 to 28 days allowed *S. enterica* levels to decrease by half. For microgreens, Cubana was less prolific at 10 CFU/g of seed, but was equivalent to Hartford once inoculation was increased by one order of magnitude. A community analysis demonstrated that the sprout rhizosphere was more species-rich compared to microgreens. Hydroponic media showed overall greater colonization by both serovars compared to either soil mixture, which is consistent with previous research by Xiao et al. (2015) and Wang and Kniel (2016).

6. Future research

Given what is currently known about bacterial and viral contamination of microgreens, many questions remain. Sunflower microgreens and pea shoots have not yet been the subject of any microbiological or viral studies, yet they are popular for producers due to the low cost of seeds, consistent germination rate, and high average fresh weight (Personal communication with beginning growers). They are also popular for beginners who may be even less attentive than established commercial operations to food safety protocols. Reed et al. (2018) and Wang and Kniel (2016) are so far the only investigators that compared multiple microgreen varieties. These as well as Gao et al. (2016) suggest that there is a species effect for both contaminant and product, though the sample sizes were small. Furthermore, most of the research into microgreen safety has been focused on bacteria, particularly *Salmonella* spp. and *E. coli*, likely due to regulatory requirements and the prevalence of these microbes in food-borne illness outbreaks. Viral contamination of microgreens should be explored further, in particular the attachment of norovirus to microgreen leaves, internalization of the virus during the growing process, and possible prevention measures. Further research on the contributions of hand harvesting versus cutting are recommended. Only Di Gioia et al. (2017) compared different types of growth media on contamination risk; these experiments need to be replicated and expanded. Additionally, earlier papers that measured AMB and coliform levels along with spoilage indicators suggested that these two factors may have an inverse relationship, though no formal correlation has been shown. Due to the short shelf life of microgreens and their tendency to be used only in small quantities, understanding the relationship between spoilage and contamination by pathogens is important.

7. Conclusion

The limited amount of data available suggests that microgreens may very well be of lower risk than sprouts in terms of food-borne illness, but the background level of bacteria is higher than that of conventional vegetables (Chandra et al., 2012; Lee et al., 2009) and is more similar to sprouts. Hydroponically grown microgreens appear to be much more susceptible to bacterial colonization compared to any solid media tested (Wang and Kniel, 2016; Xiao et al., 2015). Spoilage and shelf life may be linked to contamination by pathogens (Gao et al., 2016; Kou et al., 2013; Kou et al., 2015; Xiao et al., 2014a, 2014b). The variety of microgreen and the serovar of the contaminant may influence risk. Post-harvest washes appear so far to be ineffective and may actually increase contamination risk due to tissue damage that invites pathogens among other microorganisms (Kou et al., 2015). Pre-harvest spraying with disinfectants may provide a valid alternative the post-harvest wash for ameliorating surface contamination. Seed decontamination appears to be a critical ongoing discussion (Kou et al., 2014).

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References

Alegbeleye, O., Singleton, I., Sant'Ana, A., 2018. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. Food Microbiol. 73, 177–208. https://doi.org/10.1016/j.fm.2018.01.003.

Alum, A., Enriquez, C., Gerba, C.P., 2011. Impact of drip irrigation method, soil, and virus type on tomato and cucumber contamination. Food Environ. Virol. 3, 78–85. https://doi.org/10.1007/s12560-011-9060-y.

Aruscavage, D., Miller, S.A., Lewis Ivey, M.L., Lee, K., Lejeune, J.T., 2008. Survival and dissemination of *Escherichia coli* O157:H7 on physically and biologically damaged lettuce plants. J. Food Prot. 71, 2384–2388. https://doi.org/10.4315/0362-028X-71.12.2384.

Bartz, F.E., Lickness, J.S., Heredia, N., de Aceituno, A.F., Newman, K.L., Hodge, D.W., Jaykus, L.A., García, S., Leon, J.S., 2017. Contamination of fresh produce by microbial indicators on farms and in packing facilities: elucidation of environmental routes. Appl. Environ. Microbiol. 83, 1–10. https://doi.org/10.1128/AEM.02984-16.

Bennett, S.D., Sodha, S.V., Ayers, T.L., Lynch, M.F., Gould, L.H., Tauxe, R.V., 2018. Produce-associated foodborne disease outbreaks, USA, 1998–2013. Epidemiol. Infect. 146, 1397–1406. https://doi.org/10.1017/S0950268818001620.

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. Environ. Microbiol. 12, 2385–2397. https://doi.org/10.1111/j.1462-2920.2010.02297.x.

Bernard, H., Faber, M., Wilking, H., Haller, S., Höhle, M., Schielke, A., Ducomble, T., Siffczyk, C., Merbecks, S., Fricke, G., Hamouda, O., Stark, K., Werber, D., 2014. Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. Eur. Secur. 19. https://doi.org/10.2807/1560-7917.ES2014.19.8.20719.

Calvin, L., 2013. The Food Safety Modernization Act and the Produce Rule. Vegetables and pulses outlook. USDA Economic Research Service. VGS-353-SA2. March 29, 2013. https://www.ers.usda.gov/webdocs/publications/39535/41022_vgs353sa2.pdf?v=42125.

Canadian Food Inspection Agency, 2018a. Food Recall Warning - Goodleaf brand Daikon Radish microgreens recalled due to *Listeria monocytogenes*. (June 28, 2018). http://inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2018-06-28/eng/1530237479767/1530237483085.

Canadian Food Inspection Agency, 2018b. Updated Food Recall Warning - Evergreen Herbs brand Pea Shoots recalled due to Listeria monocytogenes. (June 7, 2018). http://inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2018-06-07/eng/1528409624622/1528409626775.

Canadian Food Inspection Agency, 2018c. Food Recall Warning - GPM brand Pea Shoots recalled due to *Listeria monocytogenes*. (June 7, 2018). http://inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2018-06-07/eng/1528383445910/1528383449409.

Castro-Rosas, J., Escartin, E.F., 2000. Survival and growth of Vibrio cholerae O1, *Salmonella typhi*, and *Escherichia coli* O157: H7 in alfalfa sprouts. J. Food Sci. 65, 162–165.

Chandra, D., Kim, J.G., Kim, Y.P., 2012. Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. Hortic. Environ. Biotechnol. 53, 32–40. https://doi.org/10.1007/s13580-012-0075-6.

Cho, H.G., Lee, S.G., Kim, W.H., Lee, J.S., Park, P.H., Cheon, D.S., Jheong, W.H., Jho, E.H., Lee, J.B., Paik, S.Y., 2014. Acute gastroenteritis outbreaks associated with ground-waterborne norovirus in South Korea during 2008–2012. Epidemiol. Infect. 142, 2604–2609. https://doi.org/10.1017/S0950268814000247.

Crowe, S.J., Mahon, B.E., Vieira, A.R., Gould, L.H., 2015. Vital signs: multistate food-borne outbreaks - United States, 2010–2014. MMWR 64, 1221–1225. https://doi.org/10.15585/mmwr.mm6443a4.

Delian, E., Chira, A., Bădulescu, L., Chira, L., 2015. Insights into microgreens physiology. Horticulture 59, 447–454. https://www.cabdirect.org/cabdirect/abstract/20153335905.

Deng, W., Gibson, K.E., 2017. Interaction of microorganisms within leafy green phyllospheres: where do human noroviruses fit in? Int. J. Food Microbiol. 258, 28–37. https://doi.org/10.1016/j.ijfoodmicro.2017.07.010.

Di Gioia, F., De Bellis, P., Mininni, C., Santamaria, P., Serio, F., 2017. Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica rapa* L.) microgreens. J. Sci. Food Agric. 97, 1212–1219. https://doi.org/10.1002/jsfa.7852.

Ding, H., Fu, T.J., Smith, M., 2013. Microbial contamination in sprouts: how effective is seed disinfection treatment? J. Food Sci. 78, R495–R501. https://doi.org/10.1111/1750-3841.12064.

Erdozain, M.S., Allen, K.J., Morley, K.A., Powell, D.A., 2013. Failures in sprouts-related risk communication. Food Control 30, 649–656. https://doi.org/10.1016/j.foodcont.2012.08.022.

European Food Safety Authority, 2013. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). EFSA J. https://doi.org/10.2903/j.efsa.2013.3025.

European Food Safety Authority, 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15 (12), 5077. https://doi.org/10.2903/j.efsa.2017.5077.

Flynn, D., 2018. Jimmy Johns pulls sprouts from all 2,727 franchise locations. Food Safety News. https://www.foodsafetynews.com/2018/01/jimmy-johns-pulls-sprouts-from-menu-at-all-2727-franchise-locations/.

Gao, X., Esseili, M.A., Lu, Z., Saif, L.J., Wang, Q., 2016. Recognition of histo-blood group antigen-like carbohydrates in lettuce by human GII.4 norovirus. Appl. Environ. Microbiol. 82, 2966–2974. https://doi.org/10.1128/AEM.04096-15.

Gelting, R., 2007. Investigation of an *Escherichia coli* O157:H7 Outbreak Associated With Dole Pre-packaged Spinach. CDC National Center for Environmental Health, pp. 1–51. https://www.cdc.gov/nceh/ehs/docs/investigation_of_an_e_coli_outbreak_associated_with_dole_pre-packaged_spinach.pdf (95899).

Gensheimer, K., Gubernot, D., 2016. 20 years of sprout-related outbreaks: FDA's investigative efforts. Open Forum Infect. Dis. 3 (1), 1438. https://doi.org/10.1093/ofid/ofw172.1140. (Poster Abstract).

Harrison, J.A., 2017. Potential food safety hazards in farmers markets. In: Food Safety for Farmers Markets: A Guide to Enhancing Safety of Local Foods. Springer International Publishing, Cham, pp. 23–37. https://doi.org/10.1007/978-3-319-66689-1_3.

Herman, K.M., Hall, A.J., Gould, L.H., 2015. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiol. Infect. 143, 3011–3021. https://doi.org/10.1017/S0950268815000047.

Hoffmann, S., Devleesschauwer, B., Aspinall, W., Cooke, R., Corrigan, T., Havelaar, A., Angulo, F., Gibb, H., Kirk, M., Lake, R., Speybroeck, N., Torgerson, P., Hald, T., 2017. Attribution of global foodborne disease to specific foods: findings from a World Health Organization structured expert elicitation. PLoS One 12. https://doi.org/10.1371/journal.pone.0183641.

Itoh, Y., Sugita-Konishi, Y., Kasuga, F., Iwaki, M., Hara-Kudo, Y., Saito, N., Noguchi, Y., Konuma, H., Kumagai, S., 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl. Environ. Microbiol. 64 (4), 1532–1535. https://www.ncbi.nlm.nih.gov/pubmed/9546189.

Jung, Y., Jang, H., Matthews, K.R., 2014. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb. Biotechnol. 7, 517–527. https://doi.org/10.1111/1751-7915.12178.

Käferstein, F.K., 2003. In: Unnevehr, L. (Ed.), Food Safety in Food Security and Food Trade, Food Safety as a Public Health Issue for Developing Countries. International Food Policy Research Institute, Washington, DC, pp. 4–6. https://ideas.repec.org/p/fpr/2020br/1002.html.
Kocharunchitt, C., Ross, T., McNeil, D.L., 2009. Use of bacteriophages as biocontrol agents to control *Salmonella* associated with seed sprouts. Int. J. Food Microbiol. 128, 453–459. https://doi.org/10.1016/J.IJFOODMICRO.2008.10.014.
Kou, L., Luo, Y., Yang, T., Xiao, Z., Turner, E.R., Lester, G.E., Wang, Q., Camp, M.J., 2013. Postharvest biology, quality and shelf life of buckwheat microgreens. Lebensm. Wiss. Technol. 51, 73–78. https://doi.org/10.1016/j.lwt.2012.11.017.
Kou, L., Yang, T., Liu, X., Luo, Y., 2014. Pre-harvest calcium application increases biomass and delays senescence of broccoli microgreens. Postharvest Biol. Technol. 87, 70–78. https://doi.org/10.1007/s10499-012-9533-5.
Kou, L., Yang, T., Liu, X., Luo, Y., 2015. Effects of pre- and postharvest calcium treatments on shelf life and postharvest quality of broccoli microgreens. Hortic. Sci. 50, 1801–1808.
Kyriacou, M.C., Rouphael, Y., Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., De Pascale, S., Santamaria, P., 2016. Micro-scale vegetable production and the rise of microgreens. Trends Food Sci. Technol. 57, 103–115. https://doi.org/10.1016/j.tifs.2016.09.005.
Lee, J.S., Kim, Ji, Park, Suhyung, 2009. Effects of chlorine wash on the quality and microbial population of 'Tah Tasai' Chinese cabbage (*Brassica campestris* var. *narinosa*) microgreen. Kor. J. Hortic. Sci. Technol. 27, 625–630.
Lin, C., Wei, C., 1997. Transfer of *Salmonella* Montevideo onto the interior surfaces of tomatoes by cutting. J. Food Prot. 60, 858–862.
Matos, A., Garland, J., 2005. Effects of community versus single strain inoculants on the biocontrol of *Salmonella* and microbial community dynamics in alfalfa sprouts. J. Food Prot. 68, 40–48. https://doi.org/10.4315/0362-028X-68.1.40.
Mir, S.A., Shah, M.A., Mir, M.M., 2017. Microgreens: production, shelf life, and bioactive components. Crit. Rev. Food Sci. Nutr. 57, 2730–2736. https://doi.org/10.1080/10408398.2016.1144557.
Moore, K., Shober, A., Hasing, G., Wiese, C., West, N., 2015. Importance of hand hygiene during the harvesting of strawberries. Hortic. Technol. 25 (3), 261–265.
Olaimat, A.N., Holley, R.A., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32, 1–19. https://doi.org/10.1016/j.fm.2012.04.016.
Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg. Infect. Dis. 19, 407–415. https://doi.org/10.3201/eid1903.111866.
Reed, E., Ferreira, C.M., Bell, R., Brown, E.W., Zheng, J., 2018. Plant-microbe and abiotic factors influencing *Salmonella* survival and growth on alfalfa sprouts and Swiss chard microgreens. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.02814-17. (AEM.02814-17).
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States-major pathogens. Emerg. Infect. Dis. 17, 7–15. https://doi.org/10.3201/eid1701.P11101.
Sivapalasingam, S., Friedman, C., Cohen, L., Tauxe, R., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67, 2342–2353. https://doi.org/10.4315/0362-028X-67.10.2342.
Solomon, E.B., Potenski, C.J., Matthews, K.R., 2002. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. J. Food Prot. 65, 673–676.
Stewart, D.S., Reineke, K.F., Ulaszek, J.M., Tortorello, M.L., 2001. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. J. Food Prot. 64, 618–622 (https://doi.org/11357873).
Stine, S.W., Song, I., Choi, C.Y., Gerba, C.P., 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. J. Food Prot. 68, 913–918. https://doi.org/10.4315/0362-028X-68.5.913.
Suslow, T.V., 2003. Key Points of Control and Management for Microbial Food Safety: Information for Growers, Packers, and Handlers of Fresh-consumed Horticultural Products. University of California Division of Agriculture and Natural Resources, Davis, CA. https://doi.org/10.3733/ucanr.8102.
Taormina, P.J., Beuchat, L.R., Slutsker, L., 1999. Infections associated with eating seed sprouts: an international concern. Emerg. Infect. Dis. 5, 626–634. https://doi.org/10.3201/eid0505.990503.
Thomas, M.K., Murray, R., Flockhart, L., Pintar, K., Pollari, F., Fazil, A., Nesbitt, A., Marshall, B., 2013. Estimates of the Burden of Foodborne Illness in Canada for 30 specified pathogens and unspecified agents. Foodborne Pathog. Dis. 10, 639–648. https://doi.org/10.1089/fpd.2012.1389.
Todd, E.C.D., Greig, J.D., Bartleson, C.A., Michaels, B.S., 2009. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. J. Food Prot. 72, 202–219. https://doi.org/10.4315/0362-028X-72.1.202.
Treadwell, D.D., Hochmuth, R., Landrum, L., Laughlin, W., 2010. Microgreens: A New Specialty Crop. University of Florida IFAS Extension (July, 1–3).
United States Department of Agriculture, 2018. Produce GAPs Harmonized Food Safety Standard. Version 1.0. Agricultural Marketing Service. January 5, 2018. https://www.ams.usda.gov/sites/default/files/media/HarmonizedStandard.pdf.
United States Department of Health Human Services, 2015. FSMA Final Rule on Produce Safety: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Final Rule. Federal Register. 80 FR 74353. pp. 74353–74568.
United States Food and Drug Administration, 1999. Microbiological safety evaluations and recommendations on sprouted seed. National Advisory Committee on Microbiological Criteria for Foods. Int. J. Food Microbiol. 52 (3), 123–153.
United States Food Drug Administration, 2016. Osage Gardens Inc. recalls Osage Gardens Organic 2oz microgreens because of possible health risk. Recalls, withdrawals, safety alerts. https://www.fda.gov/safety/recalls/ucm524638.htm.
United States Food Drug Administration, 2017a. Compliance With and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations: Guidance for Industry Draft Guidance. Draft Guid. Ind.Compliance With and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations: Guidance for Industry Draft Guidance. Draft Guid. Ind.
United States Food Drug Administration, 2017b. Chlorofields recalls Asian microgreens because of possible health risk. Recalls, recalls, withdrawals, safety alerts. https://www.fda.gov/safety/recalls/ucm548250.htm.
United States Food Drug Administration, 2018. Greenbelt Greenhouse Ltd recalls Greenbelt Microgreens brand microgreens because of possible health risk. Recalls, withdrawals, safety alerts. https://www.fda.gov/Safety/Recalls/ucm605702.htm.
Uyttendaele, M., Jaykus, L.A., Amoah, P., Chiodini, A., Cunliffe, D., Jacxsens, L., Holvoet, K., Korsten, L., Lau, M., McClure, P., Medema, G., Sampers, I., Rao Jasti, P., 2015. Microbial hazards in irrigation water: standards, norms, and testing to manage use of water in fresh produce primary production. Compr. Rev. Food Sci. Food Saf. 14, 336–356. https://doi.org/10.1111/1541-4337.12133.
Waitt, J.A., Kuhn, D.D., Welbaum, G.E., Ponder, M.A., 2014. Postharvest transfer and survival of *Salmonella enterica* serotype *enteritidis* on living lettuce. Lett. Appl. Microbiol. 58, 95–101. https://doi.org/10.1111/lam.12170.
Wang, Q., Kniel, K.E., 2016. Survival and transfer of murine norovirus within a hydroponic system during kale and mustard microgreen harvesting. Appl. Environ. Microbiol. 82, 705–713. https://doi.org/10.1128/AEM.02990-15.
Wang, M., Rong, S., Tian, P., Zhou, Y., Guan, S., Li, Q., Wang, D., 2017. Bacterial surface-displayed GII.4 human norovirus capsid proteins bound to HBGA-like molecules in Romaine lettuce. Front. Microbiol. 8, 1–9. https://doi.org/10.3389/fmicb.2017.00251.
Warriner, K., Ibrahim, F., Dickinson, M., Wright, C., Waites, W.M., 2005. Seed decontamination as an intervention step for eliminating *Escherichia coli* on salad vegetables and herbs. J. Sci. Food Agric. 85, 2307–2313. https://doi.org/10.1002/jsfa.2256.
World Health Organization, 2015. WHO Estimates of the Global Burden of Foodborne Diseases. Foodborne Disease Burden Epidemiology Reference Group, pp. 1–255.
Wright, K.M., Holden, N.J., 2018. Quantification and colonisation dynamics of *Escherichia coli* O157:H7 inoculation of microgreens species and plant growth substrates. Int. J. Food Microbiol. 273, 1–10. https://doi.org/10.1016/j.ijfoodmicro.2018.02.025.
Wuytack, E., Diels, A., Meersseman, K., Michiels, C., 2003. Decontamination of seeds for seed sprout production by high hydrostatic pressure. J. Food Prot. 66, 918–923. https://doi.org/10.4315/0362-028X-66.6.918.
Xiao, Z., 2013. Nutrition, sensory, quality and safety evaluation of a new specialty produce: microgreens. In: UMD Digital Repository, pp. 145. https://drum.lib.umd.edu/handle/1903/14900 (University of Maryland Thesis Dissertation).
Xiao, Z., Lester, G.E., Luo, Y., Wang, Q., 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. J. Agric. Food Chem. 60, 7644–7651. https://doi.org/10.1021/jf300459b.
Xiao, Z., Luo, Y., Lester, G.E., Kou, L., Yang, T., Wang, Q., 2014a. Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. Lebensm. Wiss. Technol. 55, 551–558. https://doi.org/10.1016/j.lwt.2013.09.009.
Xiao, Z., Nou, X., Luo, Y., Wang, Q., 2014b. Comparison of the growth of *Escherichia coli* O157: H7 and O104: H4 during sprouting and microgreen production from contaminated radish seeds. Food Microbiol. 44, 60–63. https://doi.org/10.1016/j.fm.2014.05.015.
Xiao, Z., Bauchan, G., Nichols-Russell, L., Luo, Y., Wang, Q., Nou, X., 2015. Proliferation of *escherichia coli* o157:h7 in soil-substitute and hydroponic microgreen production systems. J. Food Prot. 78, 1785–1790. https://doi.org/10.4315/0362-028X.JFP-15-063.