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Impact of mulches and growing season on indicator bacteria survival during lettuce cultivation

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ABSTRACT

In fresh produce production, the use of mulches as ground cover to retain moisture and control weeds is a common agricultural practice, but the influence that various mulches have on enteric pathogen survival and dispersal is unknown. The goal of this study was to assess the impact of different mulching methods on the survival of soil and epiphytic fecal indicator bacteria on organically grown lettuce during different growing seasons. Organically managed lettuce, cultivated with various ground covers — polyethylene plastic, corn-based biodegradable plastic, paper and straw mulch — and bare ground as a no-mulch control, was overhead inoculated with manure-contaminated water containing known levels of generic *Escherichia coli* and *Enterococcus* spp. Leaves and soil samples were collected at intervals over a two week period on days 0, 1, 3, 5, 7, 10 and 14, and quantitatively assessed for *E. coli*, fecal coliforms and *Enterococcus* spp. Data were analyzed using mixed models with repeated measures and an exponential decline with asymptote survival model. Indicator bacterial concentrations in the lettuce phyllosphere decreased over time under all treatments, with more rapid *E. coli* declines in the fall than in the spring ($p < 0.01$). Persistence of *E. coli* in spring was correlated with higher maximum and minimum temperatures in this season, and more regular rainfall. The survival model gave very good fits for the progression of *E. coli* concentrations in the phyllosphere over time ($R^2 = 0.88 \pm 0.12$). In the spring season, decline rates of *E. coli* counts were faster (2013 $p = 0.18$; 2014 $p < 0.005$) for the bare ground-cultivated lettuce compared to mulches. In fall 2014, the *E. coli* decline rate on paper mulch-grown lettuce was higher ($p < 0.005$). Bacteria fluctuated more, and persisted longer, in soil compared to lettuce phyllosphere, and mulch type was a factor for fecal coliform levels ($p < 0.05$), with higher counts retrieved under plastic mulches in all trials, and higher enterococci levels under straw in fall 2014 ($p < 0.05$). This study demonstrates that mulches used in lettuce production may impact the fate of enteric bacteria in soil or on lettuce, most likely in relation to soil moisture retention, and other weather-related factors, such as temperature and rainfall. The data suggest that the time between exposure to a source of enteric bacteria and harvesting of the crop is season dependent, which has implications for determining best harvest times.

1. Introduction

Agricultural practices are considered crucial factors impacting the bacteriological status of pre-harvest produce. Cropping methods are important not only for crop health and yield, but also to minimize food safety risks (Franz and van Bruggen, 2008b). Between 1998 and 2008, produce related outbreaks were responsible for the highest number of foodborne illnesses among all food commodities, with leafy greens comprising the largest produce category (Painter et al., 2013). There exists the potential for pre-harvest contamination from direct sources such as manure (Franz et al., 2008a; Johannessen, 2005) and irrigation water (Greene et al., 2008; Solomon et al., 2002). Good Agricultural Practices (GAPs) that consider methods that could introduce pathogens to the field, including soil fertilization and irrigation, are recommended to growers to minimize produce contamination risk (FDA, 2014). Assessment of pre-harvest risk, however, requires not only the identification of contamination sources, but also the interaction of these factors with cultivation practices that could affect the persistence of foodborne pathogens once introduced, and which to date, remain largely unexplored.

In Maryland, approximately 48% of farms are less than 20 ha in size (USDA, 2009) and small producers implement a variety of cropping methods. Leafy greens are more frequently irrigated by overhead application. In a study evaluating 32 leafy greens farms in the mid-Atlantic region, 81% used overhead sprinkler irrigation (Marine et al., 2015). Foliar contact with contaminated irrigation water is considered a food

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safety risk for produce, since water will contact edible portions multiple times over the growing season, and leafy greens are frequently consumed raw. In field-grown Iceberg and Romaine lettuce irrigated by various methods, overhead application with contaminated water resulted in highest levels of generic *Escherichia coli* (Fonseca et al., 2011). In mid-Atlantic and east coast states, surface water is often the primary source of irrigation water available to growers (Ragland and Tropp, 2009). In a study in New York state including 84 fresh produce growers, half reported using surface water for irrigation (Bihn et al., 2013), while in another survey in the mid-Atlantic including 313 growers, approximately half in 2010 and a quarter in 2013 reported the same (Marine et al., 2016). Surface water in this region is of inferior agricultural quality compared to groundwater (Marine et al., 2015; Pagadala et al., 2015), and may be contaminated with generic *E. coli* as well as human pathogens (Bell et al., 2015; Gu et al., 2013b; Luo et al., 2015; Micallef et al., 2012).

Mulching (use of soil covers such as plastic or straw) is a widely used cropping approach to enhance the growth of fresh produce. Mulches are applied to the soil surface for retention of soil moisture, regulation of soil temperature and suppression of weed growth. Mulching also has a positive effect on crops with improved lettuce head, leaf and stem growth, and total yields compared with bare ground (Khazaei et al., 2013; Verdial et al., 2001). Plastic mulch is the most common type and can directly affect the plant micro-climate by decreasing soil water loss and modifying the surface radiation budget (Liakatas et al., 1986), thus providing high productivity and increased nutrient accumulation in lettuce (Verdial et al., 2001). Starch-based biodegradable films (mainly from corn, potato and rice crops) have been introduced as an alternative to conventional plastic mulch to reduce environmental impact. Other biodegradable mulches include straw, hay, paper and compost. Organic mulches reduced soil temperature and maintained higher soil moisture levels compared to black plastic mulch (Schonbeck and Evanylo, 1998).

Different types of mulches have been reported to have different effects on plant pathogen risks. Straw mulch reduced levels of center rot caused by the bacterium *Pantoea ananatis* on sweet onion, while black plastic mulch had the opposite effect, speeding up the onset of the epidemic (Gitaitis et al., 2004). Fresh-market tomatoes grown using a cover bed of hairy vetch mulch or polyethylene plastic suffered markedly lower foliar disease than tomatoes grown in uncovered beds (Mills et al., 2002). Few studies have addressed mulching from a food safety perspective, and little is known about its effects on the survival of foodborne pathogens in soil and on produce. Counts of mesophilic aerobic and psychrotrophic bacteria were higher on plastic mulch-cultivated lettuce compared to lettuce grown in bare ground (Agüero et al., 2008), and splash dispersal of *Salmonella* during a simulated rain event on plastic mulch enhanced the dispersal of *Salmonella* to tomatoes (Cevallos-Cevallos et al., 2012a). To further our understanding of mulch influence on lettuce and soil microbiota, the objective of this study was to assess the effect of various mulches on the fate of soil and epiphytically-associated fecal indicator bacteria, introduced via overhead application. The indicator microorganisms tested were *E. coli*, fecal coliforms and *Enterococcus* spp. Lettuce was cultivated during two different growing seasons (spring and fall) over a two year period to additionally assess the impact of season-related effects.

2. Materials and methods

2.1. Field site and plot design

The field experiment was conducted at the Wye Research and Education Center (Wye REC) of the University of Maryland, Queenstown, MD. Experiments were conducted during the spring and fall seasons of 2013 and 2014. Bacteria were introduced via overhead application, to simulate introduction via spray irrigation, to lettuce organically grown on different mulches. There were five treatments of different ground covers: bare ground (BG) (no mulch control), black polyethylene plastic mulch (PP), biodegradable (corn-based) plastic mulch (CP), paper mulch (PM) and straw mulch (SM) (year 2 only). Plots were arranged in a randomized complete block design with four replications per treatment. Each treatment consisted of three or four 4.6 m by 0.6 m double-row beds, with 1.2 m uncovered spacing between the beds. A buffer row with polyethylene plastic mulch and planted with lettuce was laid on the southwestern side of the plot to separate it from other research fields. Twelve lettuce heads were planted 0.3 m apart in rows in each bed.

The soil at Wye was a loam soil with a pH of 5.8 to 6.2. Soils in the plot area were chisel-plowed. Raised planting beds were formed with a rototiller and bed shaper. The plots used drip irrigation and were irrigated with well water. Drip irrigation T-tape, 200 μm thick with 30 cm emitter spacing, with a flow rate of 300 LPH per 100 m tape at 0.83 bar, was buried 2.5–5.0 cm deep. One line of drip tape was used per bed and was placed midway between two rows of plants. Pelletized chicken manure pellets (Purdue AgriRecycle, Seaford, DE) were applied at a rate of 3,360 kg/ha.

2.2. Lettuce cultivation

Romaine lettuce cultivar ‘Parris Island Cos’ seeds were started in the greenhouse in 2.5 cm pots (April or September) and transplanted 30 cm apart in the field three weeks post germination. Overhead inoculum application to lettuce was performed 8 weeks post transplantation. Rainfall data were also recorded (see Section 2.6).

2.3. Inoculum preparation

To simulate lettuce contamination by overhead irrigation, water from a secondary lagoon receiving liquid dairy manure was collected from the University of Maryland's Central Maryland Research and Education Centre, Clarksville Facility (Clarksville, MD) two days before inoculation. The lagoon water was enumerated for generic *E. coli*, fecal coliforms and *Enterococcus* spp. before and after being supplemented with *E. coli* (when levels were low), and on the morning of inoculation day. Supplementation was with non-pathogenic *E. coli*, previously isolated from liquid manure from the same site, which was cultured in Tryptic Soy Broth (TSB) (BD, France) for 24 h at 37 °C to a concentration of 8 log CFU/ml and 20 ml broth was added to the inoculum (24 L). For enumeration, a serial dilution of 1:5, 1:50 and 1:500 in PBS were made up, and 100 μl of each dilution were spread for enumeration on Tryptone Bile Glucuronic Agar (TBX) plates (HiMedia Laboratories Pvt. Ltd, India) for *E. coli* and fecal coliform and Enterococcosel Agar plates (EA) (BD, Germany) for *Enterococcus* spp. The plates were incubated at 44 °C for 24–48 h (TBX) and 37 °C for 24–48 h (EA).

2.4. Overhead inoculum application and sampling

Overhead inoculation was carried out by sprinkling lagoon water of known indicator bacterial concentrations onto lettuce. In spring and fall 2013 and 2014, respectively, *E. coli* counts were 7.7 log CFU/ml, 4.2 log CFU/ml, 6.00 log CFU/ml and 6.0 log CFU/ml; fecal coliforms counts were 7.7, 3.6, 3.6 and 3.4 log CFU/ml; and *Enterococcus* spp. counts were 3.2, not measured, 1.0 and 1.6 log CFU/ml. Aliquots of 100 ml of inoculum were measured out and applied to each lettuce plant. A colander was placed over the lettuce heads to disperse the inoculum evenly over the plant. Care was taken not to sprinkle any inoculum away from the lettuce. Inoculations were performed on windless days to avoid drift. Lettuce and soil samples were collected prior to inoculation and 30 min after inoculation. Thereafter, samples were taken at 1, 3, 5, 7, 10 and 14 days post inoculation. During sampling, two to four inner leaves of lettuce were harvested randomly from separate lettuce heads within each bed using sterile scissors and collected into a labeled sterile bag. Soiled outer leaves were avoided. Simultaneously, 200 g