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Persistence of Indicator and Pathogenic Microorganisms in Broccoli following Manure Spreading and Irrigation with Fecally Contaminated Water: Field Experiment

MYLÈNE GÉNÉREUX,[1]* MARIE JO BRETON,[1] JOHN MORRIS FAIRBROTHER,[2] PHILIPPE FRAVALO,[3] AND CAROLINE CÔTÉ[1]

[1]*Research and Development Institute for the Agri-Environment (IRDA), 335 Vingt-Cinq East Road, Saint-Bruno-de-Montarville, Québec, Canada J3V 0G7; and* [2]*OIE Reference Laboratory for Escherichia coli (EcL) and* [3]*NSERC Industrial Research Chair in Meat Safety, Faculty of Veterinary Medicine, Université de Montréal, 3200 Sicotte Street, Saint-Hyacinthe, Québec, Canada J2S 2M2*

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ABSTRACT

In 2011 and 2012, trials consisting of experimental plots were carried out to evaluate the presence of pathogenic (*Listeria monocytogenes, Salmonella*) and prevalence of indicator (*Escherichia coli*) microorganisms in broccoli fertilized with liquid hog manure or mineral fertilizers and irrigated zero, one, or two times with *E. coli*–contaminated water. In 2011, results showed that *E. coli* contamination in broccoli heads was affected by the interval between irrigation and sampling ($P = 0.0236$), with a significant decrease between the first and third day following irrigation ($P = 0.0064$). In 2012, irrigation frequency significantly increased *E. coli* prevalence in broccoli samples ($P = 0.0499$). In 2012, *E. coli* counts in the soil were significantly influenced by the type of fertilizer applied, as plots receiving liquid hog manure showed higher bacterial counts ($P = 0.0006$). *L. monocytogenes* was recovered in one broccoli sample, but geno-serogrouping differentiated the isolate from those recovered in manure and irrigation water. The *L. monocytogenes* serogroup IIA, pulsotype 188 strain was found in six soil samples and in irrigation water applied 5 days before soil sampling. This study highlights the link between *E. coli* levels in irrigation water, irrigation frequency, and interval between irrigation and harvest on produce contamination. It also demonstrates that *L. monocytogenes* introduced into the soil following irrigation can persist for up to 5 days.

Fresh produce suppliers are facing growing demands to provide safe commodities, and they often participate in food safety programs, such as good agricultural practices audits. Several government agencies and industries have established recommendations or created on-farm food safety programs that include guidelines or rules regarding many steps of fruit and vegetable production, packing, and storing *(3, 4, 9, 10, 22, 53, 62, 63)*. Irrigation water and organic fertilizers have been considered as potential preharvest sources of human pathogens in crops *(44, 49, 52)*. According to the CanadaGAP food safety program, the interval between manure spreading and harvest must be greater than 120 days *(10)*. Also, in the CanadaGAP program, when irrigation water is thought to be contaminated, water analysis must be performed and *Escherichia coli* content must not exceed 100 CFU/100 ml *(10)*. However, no studies have been conducted on the combined effect of manure and irrigation.

Broccoli may be irrigated shortly before harvest, enhancing produce contamination if water quality is poor. It has also been reported that attachment of *Listeria monocytogenes* may be greater in broccoli than in other vegetables *(31)*. When grown in contaminated soil, cruciferous vegetables have shown a greater prevalence of *Salmonella* contamination than lettuce, tomatoes, and carrots *(15)*. Irregular morphology of broccoli florets enhances water retention, making this vegetable a possible substrate for bacterial contamination *(16)*. It is also often consumed raw or following minimal processing, enhancing human exposure to pathogenic microorganisms in case of contamination. Finally, broccoli is generally overhead irrigated, a process that is known to increase the risk of produce contamination compared to drip irrigation, as reported in several studies *(5, 7, 44, 49, 58, 61, 63, 67)*.

Attachment and internalization of pathogenic bacteria in plant tissues have been the subject of many studies in recent decades *(18, 20, 46, 50, 54, 61)*. Most of these studies have been carried out on cabbage and leafy greens inoculated with high bacterial concentrations, e.g., 10^4 to 10^7 CFU/g. Such high bacterial concentrations are rarely observed in agricultural fields. Also, there are very few studies *(64)* that have reported the potential impact of a combination of organic fertilizers and irrigation on microorganism persistence on produce under field conditions. The purpose of this study was to evaluate the presence of pathogenic and prevalence of indicator microorganisms in broccoli fertilized with liquid hog manure or mineral fertilizer and irrigated

* Author for correspondence. Tel: 1-450-653-7368; Fax: 1-450-653-1927; E-mail: mylene.genereux@irda.qc.ca.

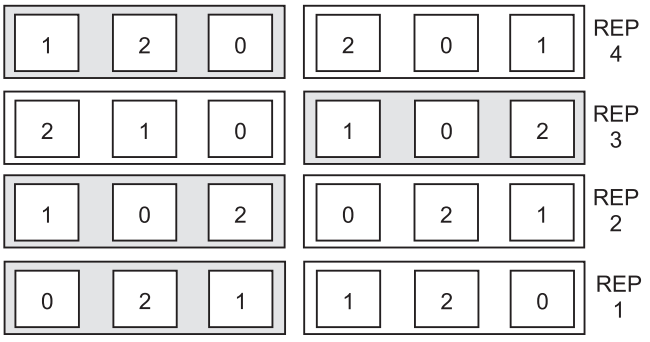


FIGURE 1. *Experimental design used in broccoli trials. Main plots fertilized with liquid hog manure are shown in gray, and plots where mineral fertilizers were applied are shown in white. Subplots were not irrigated (0), irrigated once (1), or irrigated twice (2).*

zero, one, or two times with water contaminated at *E. coli* levels found under field conditions.

MATERIALS AND METHODS

Experimental site and design. The field experiment was conducted on a 0.45-ha field located at the Research and Development Institute for the Agri-Environment experimental farm in St-Hyacinthe, Québec, Canada, in 2011 and 2012. Plots were disposed in a split-plot factorial design with six treatments repeated four times. Fertilizer type (mineral fertilizer or liquid hog manure) was the main plot factor and irrigation frequency (zero, one, or two times) was the subplot factor (Fig. 1). Plots were 4 m in length by 4 m in width and contained six rows of broccoli plants (*Brassica oleracea* L. var. *italica* cv. Everest). Plants were set 23 cm apart in the row, and rows were 76 cm apart, for 84 plants per plot in total. Replicates were set 8 m apart, and plots were 8 m apart. Finally, the whole site was fenced to minimize animal intrusion.

Fertilization. Soil was sampled before fertilization to apply adequate doses of liquid hog manure and mineral fertilizers. Each sample was composed of 15 subsamples (0- to 15-cm layers) taken in the whole field. Fertilizer application was based on soil analysis and plant requirements according to the Centre de référence en agriculture et agroalimentaire du Québec, usually used by agronomists and growers in Québec *(11)*. Soil and liquid hog manure physicochemical properties are presented in Tables 1 and 2, respectively. Urea (46-0-0), a balanced fertilizer (19-19-19), and potassium chloride (0-0-60) were used as sources of mineral nitrogen, phosphorus (P_2O_5), and potassium (K_2O). Main plots were fertilized once weekly before broccoli planting, with liquid hog manure or mineral fertilizers according to treatments. Manure was pumped from the underground holding tank of a growing-finishing hog production and spread on main plots. Doses of manure were based on P_2O_5 recommendations. Quantities needed to provide 30 kg/ha P_2O_5 were 24 m^3/ha in 2011 and 22 m^3/ha in 2012. Nitrogen and potassium needs that were not provided by manure were completed by mineral fertilizers. Shortly after spreading, all fertilizers were soil incorporated at a depth of 15 cm by using a cultivator, paying particular attention to incorporate fertilizers in mineral fertilizer treatments before slurry treatments. The interval between spreading and broccoli sampling was 68 and 61 days in 2011 and 2012, respectively.

TABLE 2. *Chemical content of liquid hog manure spread on land before broccoli transplanting*

	Chemical content (mg/kg)						
	Total N	N-NH_4	P	K	Ca	Mg	Na
2011	3,486	2,074	679	1,633	705	306	685
2012	3,342	1,741	752	1,342	897	342	693

Irrigation. Irrigation water was taken in a stone quarry and transferred into two 10,000-liter tanks. Bovine (used in both years) and pig (used in 2012 only) slurries were added to tanks to contaminate water. Slurries came from different dairy and growing-finishing hog productions. A slurry was used because it contains many strains of indicator microorganisms, such as *E. coli,* that have different survival capacities in the environment *(66)*. The ratio of pathogenic and indicator microorganism populations may also be representative of fecal contamination. Slurries from different animal species and farms were used to facilitate the tracking of the fecal contamination in broccoli samples and the soil, if required.

For treatments consisting of two irrigations, the first application was performed 18 and 19 days before broccoli sampling in 2011 and 2012, respectively (referred to as irrigation 1 hereafter). The second irrigation was performed the day before broccoli sampling in 2011 and 2012 (irrigation 2 hereafter). For treatments consisting of one irrigation application, irrigation was also carried out the day before broccoli sampling for both years (also irrigation 2 hereafter). The RainJet irrigation system (Harnois Industries, St-Thomas, Québec, Canada), which reproduces on-farm irrigation, was used to irrigate each plot individually. A water height of 25 mm, representative of growers' practices in Québec, was applied during each irrigation application, with a flow rate of 15.5 liter/min.

Sampling. Water and manure samples were taken in sterile 1-liter polypropylene bottles. Liquid manures used to contaminate irrigation water were sampled 2 days before irrigation to determine required volumes for water contamination. Bovine manure samples were taken near the pump installed in the storage tank during mixing. Hog manure samples were taken directly in the underground holding tank.

TABLE 1. *Sandy loam soil physicochemical properties in 2011 and 2012*[a]

			Mineral matter (mg/kg)										
	pH	OM (%)[b]	P	K	Ca	Mg	Al	B	Cu	Fe	Mn	Zn	Na
2011	6.38	0.98	107	81.6	751	55	531	0.149	1.06	196	13.2	2.93	3.7
2012	6.23	1.36	122	78.5	812	57	564	0.194	1.19	506	14.5	3.25	3.5

[a] Samples were from the 0- to 15-cm soil surface layers.
[b] OM, organic matter.

While water was mixed in tanks, water samples were taken at the exit of the pump bringing water to plots. These samples were collected three times during irrigation in sterile 1-liter polypropylene bottles.

Broccoli samples were taken 1, 3, and 5 days following irrigation 2. Each broccoli sample, consisting of three broccoli heads, was aseptically taken in each plot, paying particular attention to leave external broccoli rows and maintain a 50-cm buffer zone at the end of each row untouched, to minimize an edge effect. Knives were rinsed and disinfected with a 70% isopropanol solution, and disposable gloves and boots were changed between each plot to avoid cross-contamination. Broccoli heads were cut into 1-g pieces in the laboratory and homogenized to form a composite sample that was used for microbiological analyses.

Soil samples were taken in each plot 5 days following irrigation 2. They consisted of 10 hand-homogenized 15-cm-depth subsamples taken from each plot to form composite samples. Subsamples were taken with an open-ended soil probe. To reduce cross-contamination, new probes and disposable plastic boots were used for each plot.

All samples were kept at 4°C until analysis less than 24 h after sampling.

Analytical methods. *E. coli* counts in manure, broccoli, and soil samples were determined following a 3M Petrifilm *E. coli*/coliforms count plates procedure (3M Microbiology Products, St. Paul, MN) according to manufacturer's instructions and Health Canada MFHPB-34 method *(29)*. Limit of detection for this method was 10 CFU/g. *E. coli* counts in water were determined by the membrane filtration method using the modified mTEC agar (Difco, BD, Sparks, MD) according to the CEAEQ MA.700-Ec-mTEC 1.0 protocol *(12)*. Limit of detection for this method was 1 CFU/ml.

E. coli enrichment procedure was also performed for broccoli and soil samples. In brief, 225 ml of 0.1% buffered peptone water was added to 25 g of sample and incubated overnight at 35°C. One milliliter of the culture was transferred in 9 ml of Colilert medium (IDEXX Laboratories, Westbrook, ME), and tubes were water bath incubated for 24 h at 35°C. Yellow and fluorescent tubes under a 6-W, 365-nm UV light were considered as *E. coli* positive.

Presence of the *rfbE* gene (associated with *E. coli* O157), and the genes that identify the Shiga toxin–producing (stx_1, stx_2) and enteropathogenic (*eae*) *E. coli* pathotypes were analyzed as follows. Manure, broccoli, or soil samples (25 g) were added to 225 ml of modified tryptic soy broth (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37°C. For irrigation water samples, six volumes of 100 ml were filtrated on 0.45-μm porosity membranes that were placed in 75 ml of modified tryptic soy broth and incubated at 37°C for 24 h. After incubation, 1 ml of enrichment was pelleted by centrifugation, washed twice with phosphate-buffered saline (Sigma, St. Louis, MO), resuspended in 0.5 ml of sterile deionized water, and boiled for 10 min. The boiled cell suspensions were centrifuged, and the resulting cell lysates were used as DNA templates for PCR procedures based on Maluta et al. *(43)* and a protocol of the World Organisation for Animal Health Reference Laboratory for *E. coli* (Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, Québec, Canada) available at http://www.apzec.ca/en/APZEC/Protocols/APZEC_PCR_en.aspx.

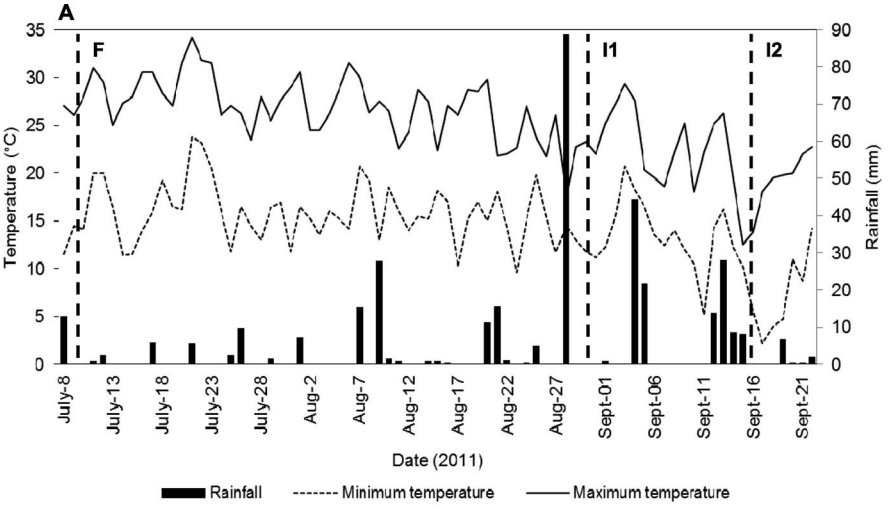
Salmonella spp. detection procedure followed Health Canada MFLP-75 method *(27)* for manure, broccoli, and soil samples and CEAEQ MA.700-Sal-PA 1.0 method *(13)* for irrigation water samples. For manure, soil, and broccoli samples, an enrichment step was performed by incubating 25 g in 225 ml of tryptic soy broth (TSB; Oxoid Ltd.) at 35°C for 24 h. For irrigation water samples, six volumes of 100 ml were filtrated, and membranes were placed in 75 ml of TSB and incubated at 35°C for 24 h. Following the preenrichment, six drops of 30 μl of enrichment were transferred on a modified semisolid Rappaport-Vassiliadis plate agar (Oxoid Ltd.) and incubated for 24 h at 42°C. When no growth was observed after 24 h, incubation was pursued for up to 72 h, including another reading after 48 h. Presumptive-positive samples (migration zone larger than 2 cm) were inoculated on a Brilliance *Salmonella* chromogenic agar plate (Oxoid Ltd.) and incubated for 24 h at 35°C. After incubation, purple colonies were confirmed by biochemical testing, using a RapID ONE system (Remel, Lenexa, KS) and agglutination with *Salmonella* O Antiserum Poly A-1 & Vi polyvalent serum (Difco, BD). Confirmed isolates were sent to the Laboratory for Foodborne Zoonoses of the Public Health Agency of Canada in Guelph, Ontario, for serotyping and phage typing.

L. monocytogenes presence was analyzed using Health Canada MFHPB-30 modified method *(28)*. For manure, soil, and broccoli samples, 25 g was aseptically transferred into 250 ml of UVM modified *Listeria* enrichment broth (Difco, BD) and incubated for 48 h at 30°C. For irrigation water samples, six volumes of 100 ml of water were filtrated, and membranes were placed into 50 ml of UVM modified *Listeria* enrichment broth and incubated for 48 h at 30°C. Then, 200 μl of incubated UVM modified *Listeria* enrichment broth was transferred into 10 ml of Fraser broth (Oxoid Ltd.) and incubated for 48 h at 37°C. Fraser selective enrichment was streaked onto Aloa agar (Aes, Combourg, France) and incubated for 48 h at 37°C. For each sample, three *L. monocytogenes*–presumptive colonies were confirmed based on hemolysis, Christie Atkins Munch-Petersen test, motility in semisolid agar, and carbohydrate use in broths (xylose, rhamnose, mannitol) (Difco, BD). Genus and species of each isolate were further confirmed by amplification of the *prs* and *prfA* genes, respectively, and geno-serogrouping of the isolates was done using the multiplex PCR protocol described by Kerouanton et al. *(36)*. Pulsotypes were determined by the Laboratoire de santé publique du Québec, according to the Centers for Disease Control and Prevention Pulse-Net protocol for *L. monocytogenes (24)*. *Asc*I and *Apa*I were used to cleave the bacterial DNA, and migrations were done in a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA). Restriction patterns were compared using BioNumerics software version 6.5 (Applied Maths, Kortrijk, Belgium) using the Dice correlation, with a position tolerance of 1% *(21)*.

For each method of quantification, a method blank using sterile media and a positive control using a dilution containing *E. coli* (ATCC 25922) were performed for each sample lot. For enrichment procedures, a method blank and a negative, a positive, and a matrix control were properly performed for each sample lot. Strains used in negative controls were *E. coli* (ATCC 25922) for *Salmonella* and pathogenic *E. coli* methods, *Klebsiella pneumoniae* (ATCC 13833) for the *E. coli* method, and *Listeria innocua* (ATCC 43895) for the *L. monocytogenes* method. Matrix controls consisted of an additional 25 g of sample containing positive strains and processed as other samples.

Climate data. Precipitation and air temperature data were provided by AgWeather Quebec, a public weather service initiated by federal and provincial governments in Canada. The daily minimum and maximum air temperature and rainfall for 2011 and 2012 are presented in Figure 2.

Statistical analysis. A generalized linear mixed model using SAS GLIMMIX procedure (SAS Institute Inc., Cary, NC) was



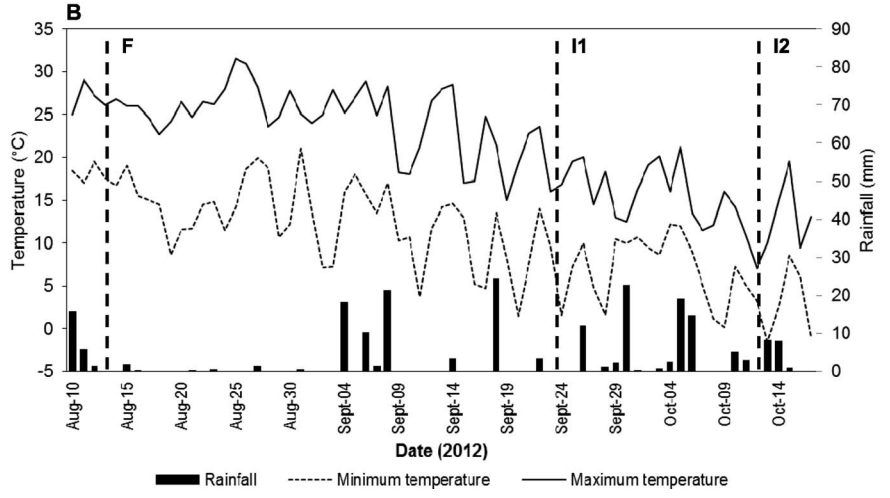


FIGURE 2. *Minimum and maximum air temperature and rainfall in 2011 (A) and 2012 (B). Fertilization (F), irrigation 1 (I1), and irrigation 2 (I2) are indicated by dotted lines. Broccoli heads were sampled 1, 3, and 5 days following irrigation 2.*

performed to evaluate the impact of the treatments on *E. coli* prevalence in broccoli and in soil samples. This model takes into account the Poisson distribution of the observations (*E. coli* counts in soil samples), the binomial distribution of the observations (*E. coli* presence or absence in broccoli samples), and the random effects of years, plots, subplots, and subsamples within subplots, as described in Littell et al. *(41)*. Fixed effects of irrigation, interval between irrigation and sampling, fertilizer, and interaction irrigation × fertilization were tested for significance with *F* tests, and treatment mean counts were calculated and compared using contrasts.

RESULTS AND DISCUSSION

Liquid hog manure used for spreading. Mean *E. coli* counts in liquid hog manure were 5.6 log CFU/g in 2011 and 5.2 log CFU/g in 2012. *Salmonella* serovar Typhimurium DT 104 was detected in liquid hog manure spread to plots in 2011, but not in 2012. No *L. monocytogenes* or *E. coli* carrying *rfbE, stx*$_1$, *stx*$_2$, and *eae* genes were detected in slurry used for fertilization.

Slurry-contaminated irrigation water. *E. coli* counts in manure used for water contamination and irrigation water samples are shown in Table 3. In 2011, *Salmonella* (serovar Schwarzengrund) was found in bovine manure used for irrigation 2, as well as in water (serovar Derby). Two hypotheses may explain why different serovars were detected. First, manure can harbor many serovars that may not all be recovered during analysis. Jokinen et al. *(35)* reported that 50 and 10% of *Salmonella*-positive fecal samples taken from cows and pigs, respectively, contained more than one *Salmonella* serovar. Also, water from the

TABLE 3. E. coli *counts in liquid manures and contaminated irrigation water in 2011 and 2012*

	2011		2012	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
Liquid bovine manure (log CFU/g)	3.9	3.5	4.8	4.9
Liquid hog manure (log CFU/g)	Not used	Not used	3.7	4.9
Irrigation water (log CFU/100 ml)	2.6	2.7	3.5	3.5

TABLE 4. *Impact of fertilizer, irrigation frequency, and irrigation-sampling interval on* E. coli *prevalence in broccoli heads in 2011 and 2012*[a]

Parameter	2011		2012	
	df	Pr > *F*	df	Pr > *F*
Fertilizer	1	0.1818	1	0.2797
Irrigation frequency	2	0.8909	2	0.0499
Irrigation-sampling interval	2	0.0236	2	0.2124

[a] df, degrees of freedom; Pr > *F*, *P* value of the *F* test.

stone quarry could have been contaminated by environmental sources such as wildlife, especially birds. *Salmonella* serovar Derby, which was found in irrigation water, has been reported in gulls by Duartea et al. *(17),* but it is mainly associated with swine *(51). L. monocytogenes* and *E. coli* carrying *rfbE, stx*$_1$, *stx*$_2$, and *eae* genes were not detected in liquid manure and irrigation water sampled in 2011.

In 2012, *Salmonella* and *E. coli* carrying *rfbE, stx*$_1$, *stx*$_2$, and *eae* genes were not detected in manure and irrigation water. However, *L. monocytogenes* was found in bovine manure used to contaminate water used for both irrigations, and in water from irrigation 2. Strains were characterized as belonging to serogroup IVB, pulsotype 196, and serogroup IIA, pulsotype 188, respectively.

Broccoli. Results from seasons 2011 and 2012 were statistically analyzed separately because *E. coli* counts in irrigation water were different. In fact, mean *E. coli* contents in water applied in 2011 and 2012 were 2.7 and 3.5 log CFU/100 ml, respectively. *E. coli* counts could not be determined in any broccoli samples by using the enumeration method ($n = 144$) (limit of detection = 10 CFU/g). Following enrichment, an overall *E. coli* prevalence of 15 and 51% was observed in broccoli samples taken in 2011 and 2012, respectively. The finding of an *E. coli* level of 3.5 log CFU/100 ml in irrigation water applied in 2012 may explain differences between the trials. The impacts of fertilizer, irrigation frequency, and sampling day on *E. coli* prevalence in broccoli samples are shown in Table 4.

In 2011, the interval between irrigation and broccoli sampling had a significant impact on *E. coli* prevalence ($P = 0.0236$). In fact, a rapid decline of *E. coli* prevalence was observed in broccoli heads during the sampling period, reaching undetectable levels 5 days after irrigation (Fig. 3). A statistically significant difference was observed in samples taken on the first day following irrigation compared with samples taken 3 days following irrigation ($P = 0.0064$). Fertilizer and irrigation frequency showed no statistically significant impact on *E. coli* prevalence in broccoli samples; however, *E. coli* was more frequently detected in irrigated than in nonirrigated broccoli heads. Indeed, *E. coli* prevalence in broccoli samples irrigated zero, one, and two times was 0, 21, and 25%, respectively. *E. coli* was also more frequently recovered in broccoli samples fertilized with slurry than in those fertilized with mineral fertilizers, with prevalence reaching 19 and 11%, respectively (not statistically significant). No pathogenic bacteria or genes

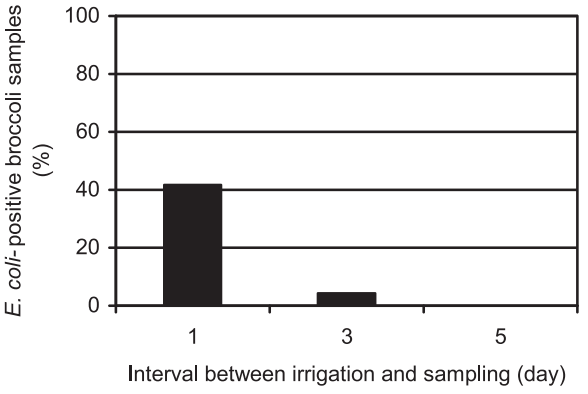


FIGURE 3. E. coli *prevalence in broccoli samples according to interval between irrigation and sampling in 2011.*

associated with pathogenic *E. coli* were recovered in broccoli samples in 2011.

In 2012, irrigation frequency had a statistically significant impact on *E. coli* contamination ($P = 0.0499$) (Fig. 4). The high *E. coli* level in irrigation water may explain this observation. The risk to recover *E. coli* in broccoli samples was 5.4-fold higher in plots that received two irrigations compared with those receiving no irrigation ($P = 0.0195$) and it was 3.7-fold higher in broccoli heads irrigated once instead of nonirrigated ($P = 0.0611$). *E. coli* prevalence in broccoli heads fertilized with slurry or mineral fertilizers was 56 and 47%, respectively. *E. coli* prevalence in broccoli heads reached 67, 42, and 46% for sampling days 1, 3, and 5, respectively (not statistically significant).

No *Salmonella* or *E. coli* carrying *rfbE, stx*$_1$, *stx*$_2$, and *eae* genes were detected in broccoli samples in 2012. One broccoli sample was found positive for *L. monocytogenes* in 2012 (serogroup IIa, pulsotype 394). Genetic characterization demonstrated a pulsotype that was not previously encountered during surveillance of human listeriosis in Québec. This strain was taken 1 day following irrigation in a manure-fertilized plot that received one irrigation application. Water for this irrigation had also been found positive for *L. monocytogenes,* although a different pulsotype was observed (serogroup IIA, pulsotype 188). Therefore, no link was established between irrigation and presence of *L. monocytogenes* in broccoli samples. Since pathogenic

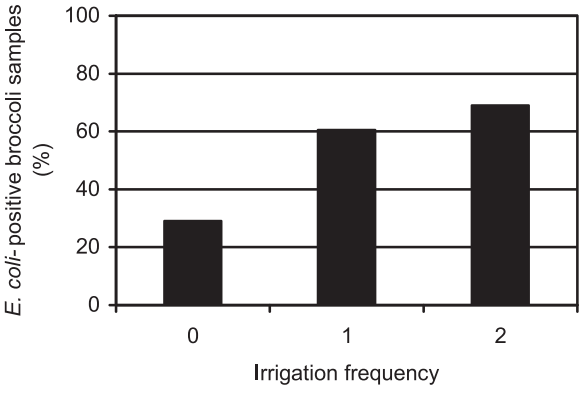


FIGURE 4. E. coli *prevalence in broccoli samples according to irrigation frequency in 2012.*

bacteria have been detected in only one broccoli sample, it is difficult to discuss the reliability of *E. coli* as an indicator microorganism in the context of this study.

Several studies have reported occurrence of indicator and pathogenic microorganisms in fresh produce or minimally processed vegetables in packaging facilities and in retail establishments *(1, 23, 33, 34, 55),* but broccoli was not sampled in these studies. In fact, few data have been reported on broccoli, especially throughout production and at harvest. Thunberg et al. *(65)* reported the absence of *E. coli, Listeria, Campylobacter,* and *Salmonella* in broccoli samples collected from retail supermarkets or farmer's markets in the Washington, DC, area. Ryu et al. *(57)* reported *E. coli* counts of less than 1 log CFU/g in broccoli samples taken in retail markets in Korea. Mukherjee et al. *(48)* reported an *E. coli* prevalence of 11% in broccoli samples taken in the field at harvest. However, few samples were taken in these studies: 13 *(65),* 30 *(57),* and 36 *(48)* samples. Many factors can affect bacterial survival on fruits and vegetables at the preharvest stage, including UV exposure *(47, 62),* temperature *(25),* and produce surface drying *(62).* It is also known that produce may contain essential oils, antioxidants, and antimicrobial components that have an impact on microorganisms *(59).* Such compounds are naturally present in broccoli, and they may explain the low *E. coli* prevalence observed in this study. However, these components have not been quantified. Several studies have reported the antimicrobial properties of glucosinolates and isothiocyanates (ITCs) on indicator and pathogenic microorganisms *(2, 6, 32, 37, 40, 68),* including studies made on fresh cruciferous vegetables *(8, 26, 30, 38, 39, 60).* Glucosinolates are a class of organic compounds that occur as secondary metabolites in almost all *Brassicaeae,* for which antibacterial activity has been recognized for many decades *(2).* They are present in the cell vacuole and are known to play a role in cell defense, being converted to compounds such as ITCs following injury to the plant *(2).* Sulforaphane and iberin are major ITCs in broccoli and are found in high concentrations in heads or inflorescences *(56).* Wilson et al. *(68)* also reported the presence of 2-phenylethyl ITC in broccoli. Rodríguez-Hernández et al. *(56)* have concluded that ITC concentration depends on the cultivar, plant organ, and sample processing.

The present experiment was conducted from July to September 2011 and from August to October 2012; average temperature from fertilization until the last sampling was 19.8 and 15.7°C in 2011 and 2012, respectively. Rain accumulation over the same periods reached 337.2 and 186 mm in 2011 and 2012, respectively. Glucosinolate content in cruciferous vegetables is also influenced by climatic conditions. Ciska et al. *(14)* have reported that low average 10-day rainfall and high average temperature during the vegetation period resulted in a significantly increased glucosinolate content in 11 cruciferous vegetables. In our study, average temperature and rainfall accumulation were lower in 2012 compared with values in 2011, but *E. coli* was more often detected in 2012 broccoli samples (51%), compared with 2011 broccoli samples (15%). Since climatic conditions were not the only varying factor between years, it is difficult to draw conclusions on the impact climate conditions may have on *E. coli* prevalence and persistence in fields.

Soil. In 2011, *E. coli* counts could be determined in one soil sample (10 CFU/g) taken in a mineral-fertilized plot that received one irrigation ($n = 24$). Following enrichment, 100% *E. coli* prevalence in manure-fertilized soil samples and 75% in mineral-fertilized plots ($n = 24$) was observed. No link was established between bacterial prevalence in soil samples and irrigation frequency. *E. coli* was detected in two, three, and four of four plots irrigated zero, one, and two times, respectively, but differences were not statistically significant. No *L. monocytogenes, Salmonella,* or pathogenic *E. coli* was detected in soil samples in 2011.

In 2012, 10 soil samples presented countable *E. coli* populations ($n = 24$). Nine samples were from manure-fertilized plots that received no (3 samples), one (3 samples), or two (3 samples) irrigations. The other positive sample came from a mineral-fertilized plot that received one irrigation application. Statistical analysis was performed only on data generated in 2012, because the number of samples with countable *E. coli* was too low in 2011. Mean *E. coli* counts in soil samples taken in manure-fertilized plots (36 CFU/g) were statistically different from those in plots that received mineral fertilizers (1 CFU/g) ($P = 0.0006$). Following the enrichment procedure, a high *E. coli* prevalence was observed in soil samples, both from manure-fertilized plots (100% prevalent) and mineral-fertilized plots (92% prevalent) ($n = 24$). No link was established between bacterial prevalence in soil samples and irrigation frequency. *E. coli* was detected in three, four, and four of four plots irrigated zero, one, and two times, respectively. In addition, even though *E. coli* prevalence in soil samples was high, no link was established between soil and broccoli samples.

Six soil samples were positive for *L. monocytogenes* in 2012, including two and four samples taken in slurry- and mineral-fertilized plots, respectively. All samples originated in plots that received two irrigations, and *L. monocytogenes* isolates presented the same genetic profile as those recovered from irrigation water taken from the second irrigation, i.e., serogroup IIA, pulsotype 188. This confirms that *L. monocytogenes* recovered in soil was from irrigation water and that it can persist at least 5 days in soil after irrigation. Strawn et al. *(64)* reported that irrigation within 3 days of sample collection increased the likelihood of an *L. monocytogenes*–positive field. It has also been reported that survival of enteric pathogens may be affected by soil texture *(42)* and temperature *(45).* For example, *L. monocytogenes* and *E. coli* O157:H7 survival time was considerably greater in clay and loam soils because of their greater microporosity and moisture content *(19).* In the present study, plots were set up on a sandy loam soil presenting lower water retention potential than clay soils and therefore inducing less microbial persistence. *L. monocytogenes* survival is also increased when soil temperature is low. McLaughlin et al. *(45)* reported undetectable levels of *L. monocytogenes* in soils kept at 25 and 30°C after 14 days, whereas bacteria were still present in soil maintained at 8°C. Average air

temperature between irrigation 2 and soil sampling was 8.5°C, potentially improving bacterial survival. No *Salmonella* or *E. coli* carrying *rfbE, stx*$_1$, *stx*$_2$, and *eae* genes was detected in soil samples in 2012.

This study emphasizes the impacts that quality of water used for irrigation and interval between irrigation and harvest have as potential factors in broccoli contamination at the farm scale. In addition, climatic conditions, irrigation frequency, and starting *E. coli* contamination levels in water may influence the risk of broccoli contamination. Because no interaction was observed between manure application and irrigation frequency on *E. coli* prevalence on broccoli heads at harvest, it is hard to draw conclusions on the combined effect of both factors on produce contamination. Finally, this study demonstrates that irrigation water may introduce *L. monocytogenes* into the soil, and it may persist 5 days. This interval between irrigation and harvest is commonly observed in broccoli production, thus enhancing the risk of contamination if vegetables come in contact with soil particles.

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