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Evaluation of Fertilization-to-Planting and Fertilization-to-Harvest Intervals for Safe Use of Noncomposted Bovine Manure in Wisconsin Vegetable Production

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ABSTRACT

Fresh bovine manure was mechanically incorporated into loamy sand and silty clay loam Wisconsin soils in April 2004. At varying fertilization-to-planting intervals, radish, lettuce, and carrot seeds were planted; crops were harvested 90, 100, 110 or 111, and 120 days after manure application. As an indicator of potential contamination with fecal pathogens, levels of *Escherichia coli* in the manure-fertilized soil and presence of *E. coli* on harvested vegetables were monitored. From initial levels of 4.0 to 4.2 log CFU/g, *E. coli* levels in both manure-fertilized soils decreased by 2.4 to 2.5 log CFU/g during the first 7 weeks. However, *E. coli* was consistently detected from enriched soil samples through week 17, perhaps as a result of contamination by birds and other wildlife. In the higher clay silty clay loam soil, the fertilization-to-planting interval affected the prevalence of *E. coli* on lettuce but not on radishes and carrots. Root crop contamination was consistent across different fertilization-to-harvest intervals in silty clay loam, including the National Organic Program minimum fertilization-to-harvest interval of 120 days. However, lettuce contamination in silty clay loam was significantly ($P < 0.10$) affected by fertilization-to-harvest interval. Increasing the fertilization-to-planting interval in the lower clay loamy sand soil decreased the prevalence of *E. coli* on root crops. The fertilization-to-harvest interval had no clear effect on vegetable contamination in loamy sand. Overall, these results do not provide grounds for reducing the National Organic Program minimum fertilization-to-harvest interval from the current 120-day standard.

Bovine manure provides macronutrients and micronutrients needed by growing plants, and thus it is an important fertilizer *(20)*, particularly for organic farmers. However, bovine manure may contain a variety of pathogenic bacteria *(18, 19, 28, 39, 40)* that may contaminate vegetables grown in manure-fertilized soils and subsequently cause foodborne illness. Treating manure to reduce pathogen numbers prior to its use as fertilizer and applying manure to land far in advance of planting or harvest to maximize pathogen death in the manure-fertilized soil environment are two possible approaches to reducing the risk of contamination. Composting is an accepted manure pathogen-reduction treatment *(36)*, but complete destruction of pathogens is not necessarily assured by this technique *(4, 12, 17, 22)*. Passive manure storage will also result in pathogen death *(15)*. Death of manure-borne pathogens also occurs when the manure is incorporated into the soil, although weather conditions, desiccation, soil type, protozoan populations, and degree of manure incorporation are all likely to affect pathogen survival *(2, 3, 5, 6, 10, 11, 13, 21, 25, 29, 32–34, 37)*.

To allow for a wide variety of soil, environmental, and manure handling practices, the U.S. Department of Agriculture (USDA) National Organic Program (NOP) specifies a minimum manure fertilization-to-harvest interval to provide adequate assurance of safety. Specifically, the NOP regulations require that at least 120 days elapse between application of noncomposted manure and the harvest of organic crops that have edible portions exposed to soil particles *(36)*. Earlier studies using pathogenic bacteria in controlled-environment chambers and soil beds suggested that a fertilization-to-harvest interval of less than 120 days might be safe in certain Wisconsin situations *(23)*. These studies, along with the work of others, validated the use of indigenous *Escherichia coli* as a surrogate for pathogenic *E. coli* O157:H7 *(25)* and *Salmonella (23)*. The results of a 2003 garden-scale field study *(16)* suggested that (i) adherence to the NOP minimum 120-day fertilization-to-harvest interval would not ensure the absence of fecal bacteria on vegetables grown in three Wisconsin soils fertilized with noncomposted bovine manure, (ii) recontamination of soil via wildlife feces makes absolute prevention of contamination unlikely, and (iii) decreasing the 120-day fertilization-to-harvest interval in Wisconsin to 100 days would only slightly increase the risk of contamination. Further, a review of literature on pathogen colonization of vegetable seedlings suggested that the fertilization-to-planting interval could also be a significant factor influencing the likelihood of contamination *(8, 9)*.

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In the present study, conducted in the spring and summer of 2004, we expanded upon the 2003 field study by using mechanical application of manure, mechanical tillage, and a common manure application date at each of two field sites to negate the problem of plot-to-plot traffic of wildlife. In order to evaluate the effect of fertilization-to-planting and fertilization-to-harvest intervals on the prevalence of *E. coli* on vegetables, we staggered vegetable planting and harvest dates. The specific objectives of the study were (i) to determine whether the NOP minimum 120-day fertilization-to-harvest interval could be decreased under Wisconsin conditions without significantly increasing *E. coli* prevalence on vegetables and (ii) to determine the extent to which the fertilization-to-planting and fertilization-to-harvest intervals affected the prevalence of *E. coli* on vegetables.

MATERIALS AND METHODS

Field sites and weather conditions. The field study was conducted at University of Wisconsin–Madison Agricultural Research Stations at Hancock and West Madison, Wis., during the spring and summer of 2004. Typical soil physical characteristics for each field site (as previously determined by analyses at the University of Wisconsin–Madison Soil and Plant Analysis Laboratory) are 87% sand, 6% silt, and 7% clay for the loamy sand (LS) soil at Hancock, and 19% sand, 65% silt, and 16% clay for the silty clay loam (SCL) soil at West Madison. The soil pH prior to the start of the 2004 study was 6.8 for LS and 6.3 for SCL (analysis by University of Wisconsin–Madison Soil and Plant Analysis Laboratory). Compared with 1994 to 2003 averages, spring–summer 2004 was characterized by unusually abundant rainfall (Table 1). Prior to the study, each field was cropped in alfalfa during the 2002 growing season. A nonselective, broad-spectrum herbicide (RoundUp Ultra, Monsanto, St. Louis, Mo.) was applied to clear the field in fall 2002, and then the sites were used in the 2003 field study *(16).* At the conclusion of the 2003 study at both sites, four strips approximately 36.9 m long by 3.1 m wide were again sprayed with nonselective, broad-spectrum herbicide and tilled. Between each strip, there was a 4.6-m alley containing white clover planted in spring 2003. At each field site, one strip served as a control, with manure applied to half of the strip's length but no vegetables planted, and no manure applied to the other half of the strip with carrots planted in one-fourth of the strip's length. One vegetable type was assigned to each of the remaining three strips at each site (Fig. 1).

Bovine manure application. Fresh manure (≤3 days old) was collected at the University of Wisconsin–Madison Dairy Cattle Instruction and Research Center from a herd of lactating Holstein cows fed a standard corn- and soy-based diet. Manure was applied at an approximate rate of 67.2 metric tons (wet weight) per ha (a typical rate for Wisconsin farming) using a conventional manure spreader. The manure was then mechanically incorporated to an approximate depth of 15 cm. Manure application occurred on 13 April (LS) and 14 April (SCL).

Vegetable production. In each noncontrol strip, one type of vegetable was planted. Four plantings were done approximately 10 days apart to study four different fertilization-to-planting intervals. Planting dates were also chosen so that crops would be mature between 90 and 120 days after fertilization (Fig. 1). Vegetables were planted using 4.6-m seed tapes evenly spaced in the strip. The varieties of vegetables planted were Nantes Half-Long carrots (W. Atlee Burpee & Co., Warminster, Pa.), Cherry Belle radish (Burpee), and Black-seeded Simpson lettuce (Burpee). Immediately prior to planting radishes in LS, chlorpyrifos (Lorsban 15 G, Dow Agrosciences, Indianapolis, Ind.) was applied to control cabbage maggot (*Delia radicum*). The application was in-furrow at a depth of 2.5 cm, at a rate of 30.3 g/100 furrow meters. Weeds were controlled by a combination of mechanical tillage, manual hoeing, and manual removal. The vegetable plots on LS were irrigated from the week of 23 June through the week of 18 August, at a rate of either 1.27 or 2.54 cm/week (Table 1). No irrigation was necessary on the SCL strips.

Soil sampling and analysis. Soil samples were analyzed at weekly intervals from each strip at each field site, beginning at the time of manure application. Prior to planting, each noncontrol strip was divided into a 6 by 6 grid of 36 equal-area units (6.15 by 0.5 m) and a composite of three soil core samples was collected from three randomly selected sets of grid coordinates. When a vegetable planting occurred, three samples were taken from randomly selected sites in a 0.6-m zone surrounding the seed tape and the remaining unplanted strip was divided into a new 6 by 6 grid of equal-area units and three samples taken. In each unplanted quarter of the control strip, three samples were taken as described above. Once carrots were planted in the fourth quarter of the control strip, three samples were taken from the 0.6-m zone surrounding the seed tape. In the two quarters of the control strip to which no manure was applied, single-use boot covers were worn while sampling to prevent contamination of the soil. Each soil sample consisted of three soil cores (approximately 10 cm deep) collected using one sterile aluminum soil corer (Forestry Suppliers, Jackson, Miss.), a sterile tongue depressor to push soil out of the corer, and a sterile stomacher filter bag (Nasco, Ft. Atkinson, Wis.). Soil samples were placed in shaded insulated coolers, transported back to the laboratory, and refrigerated (5°C) until analysis. Soil analysis steps are summarized in Figure 2. Each soil sample was weighed, and then 198 ml of Butterfield's phosphate diluent (Nelson Jameson, Marshfield, Wis.) was added to the entire sample (usually 180 to 220 g, with ranges of 139 to 238 g for LS and 120 to 281 g for SCL). The diluted sample was then manually shaken for 30 s, set still for 30 s, and then manually shaken for another 30 s. Decimal serial dilutions in Butterfield's phosphate diluent were prepared, and 1.0 ml of a given dilution was plated on a corresponding 3M Petrifilm *E. coli*–coliform count plate (3M Microbiology Products, St. Paul, Minn.). The initial sample dilution (in the stomacher bag) was not plated because the soil color obscured colonies on the Petrifilm *E. coli*–coliform count plate. Plates were incubated at 35°C for 48 h, and then presumptive *E. coli* colonies (blue with associated gas) were counted. With the soil sample weight, dilution factor, and number of presumptive colonies, the log CFU per gram of soil was calculated for each sample and then the mean log CFU per gram value for each area was calculated from log CFU per gram of each sample within the area. When no colonies were detected for the least dilute sample plated, a log CFU per gram value was calculated by subtracting 0.1 from the detection limit. With the typical sample size and plating protocol, the detection limit was 1.3 log CFU/g.

When levels of *E. coli* had sufficiently decreased, an enrichment procedure was used in addition to the direct plating procedure. Additional samples were taken from each randomly selected location for enrichment. In this procedure, the initial dilution was performed using nutrient broth (Difco, Becton Dickinson, Sparks, Md.) instead of Butterfield's phosphate diluent. For a nonselective enrichment, the initial dilution was incubated 24 h at 35°C. Then the initial dilution was gently mixed and 1.0 ml was transferred

TABLE 1. *Average maximum temperature, average minimum temperature, amount of rain, amount of irrigation (loamy sand—Hancock Agricultural Research Station only) at field sites during spring and summer of 2004*

Week ending	Average maximum (°C)			Average minimum (°C)			Cumulative rain (cm)			Irrigation (cm)
	2004	2003	1994–2003 avg	2004	2003	1994–2003 avg	2004	2003	1994–2003 avg	2004
Loamy Sand—Hancock Agricultural Research Station										
7 April	14	3	9	−1	−5	−2	0	0.79	1.55	—
14 April	9	13	12	−2	−1	0	0.07	0.79	3.56	—
21 April	18	12	13	6	2	2	2.64	3.94	6.48	—
28 April	14	18	17	3	3	2	3.25	5.46	8.00	—
5 May	16	14	17	3	5	5	3.78	9.07	10.82	—
12 May	22	18	18	16	7	7	9.75	16.28	13.46	—
19 May	18	19	20	12	8	7	12.98	17.68	15.11	—
26 May	17	19	20	13	4	7	18.24	17.68	16.25	—
2 June	19	22	23	9	8	9	22.15	18.82	19.15	—
9 June	25	21	22	14	11	11	26.93	23.65	21.44	—
16 June	23	24	24	13	12	13	40.16	24.11	24.72	—
23 June	22	27	27	9	12	15	41.18	24.47	29.85	1.27
30 June	22	25	27	10	13	15	41.54	26.40	32.52	1.27
7 July	22	29	26	14	17	15	46.37	28.23	35.21	1.27
14 July	26	23	27	15	14	15	46.95	31.05	37.06	1.27
21 July	28	27	27	17	13	16	47.53	31.05	41.73	2.54
28 July	26	27	27	13	15	15	47.53	31.05	43.41	2.54
4 August	27	27	27	16	15	15	51.72	32.92	45.37	2.54
11 August	22	27	26	12	14	15	52.02	32.92	47.10	2.54
18 August	23	30	25	9	17	14	52.81	32.92	50.68	2.54
Total							52.81	32.92	50.68	16.54
Silty Clay Loam—West Madison Agricultural Research Station										
7 April	14	3	10	1	−2	1	0	0.89	1.19	
14 April	11	16	15	−2	0	3	0	0.89	3.77	
21 April	20	15	14	8	5	5	3.45	2.44	6.82	
28 April	14	18	17	3	4	5	4.34	2.44	8.79	
5 May	16	14	17	4	6	7	4.47	4.73	10.70	
12 May	22	17	18	11	9	8	7.80	9.76	14.10	
19 May	18	19	20	7	9	9	12.40	10.83	16.45	
26 May	20	19	20	10	7	9	26.90	12.48	18.45	
2 June	19	21	22	11	9	11	31.90	14.00	22.26	
9 June	26	20	21	14	12	11	31.90	15.78	25.58	
16 June	24	24	24	15	14	14	36.09	15.78	29.15	
23 June	21	27	27	12	13	16	38.66	20.03	32.93	
30 June	22	26	27	11	15	17	40.59	23.94	35.33	
7 July	24	30	27	15	18	17	44.76	29.15	37.96	
14 July	26	23	27	15	15	16	45.95	30.19	39.36	
21 July	26	26	28	16	15	18	49.81	33.36	42.98	
28 July	24	26	27	14	17	17	51.08	34.53	44.57	
4 August	26	26	28	18	16	17	55.98	34.66	48.12	
11 August	22	27	26	12	14	15	56.44	34.66	50.60	
18 August	23	30	25	9	17	14	57.43	34.66	52.36	
Total							57.43	34.66	52.36	

to 9 ml of lauryl tryptose broth (Difco), which was vortex mixed and incubated at 45.5°C for 24 h as a selective enrichment step. Next, the contents of the selective enrichment tube were vortex mixed and one loopful was streaked to Levine-eosin methylene blue agar (Difco). Each Levine-eosin methylene blue agar plate was incubated 24 h at 35°C and observed for colonies with dark centers, with or without associated metallic sheen. A positive result was recorded if at least one such colony was present. For presumptive-positive samples, a typical colony was tested to confirm its identity as *E. coli.* One typical colony was selected from a presumptive *E. coli*–positive sample for each vegetable-growing area within a strip. Similarly, one typical colony was selected from a presumptive *E. coli*–positive sample for each soil sampling area. For confirmation, presumptive colonies from Petrifilm *E. coli*–coliform count and Levine-eosin methylene blue agar plates were streaked to brain heart infusion agar (Difco) and incubated 24 h at 35°C, and then an isolated colony was tested for cell morphology, gram reaction, oxidase reaction, and biochemical characteristics (API 20E kit, bioMérieux, Hazelwood, Mo.). Over the course of the study, 77% of presumptive *E. coli* isolates from

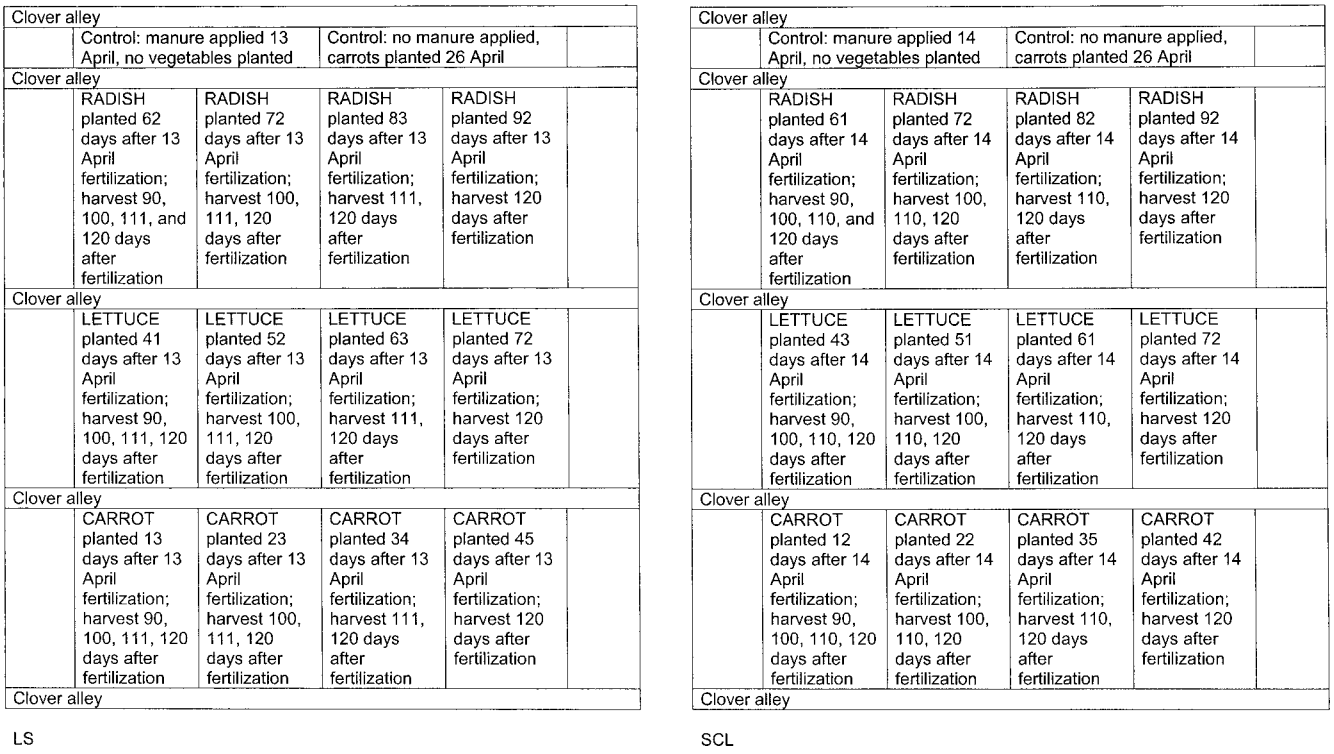

FIGURE 1. *Diagram of field plots for loamy sand (LS) and silty clay loam (SCL) sites with dates of manure application, fertilization-to-planting intervals, and fertilization-to-harvest intervals shown.*

directly plated LS samples, 88% of isolates from enriched LS samples, 57% of isolates from directly plated SCL, and 93% of isolates from enriched SCL were confirmed as *E. coli.*

Vegetable sampling and analysis. Vegetable samples were obtained at harvest. Fertilization-to-harvest intervals for which samples were obtained are listed in Figure 1. From a randomly selected location along the seed tape, a sample consisted of either two radishes or two carrots from which the stems and leaves were removed using scissors previously sprayed with 70% (vol/vol) ethanol or four mature leaves of lettuce cut using sanitized scissors and handled using sanitized latex gloves. At harvest, leaves were not contacting the soil surface. On occasion, however, soil contact via rain splashing had visibly occurred. Thus, detected *E. coli* could reflect direct soil contact, direct or indirect contact with bird or animal feces, or internalization of *E. coli.* Vegetable analysis steps are summarized in Figure 2. Vegetable samples were placed in sterile stomacher filter bags and transported to the laboratory in insulated coolers. In the laboratory, a piece of cheesecloth was placed in a colander that had been previously sprayed with 70% ethanol. Each sample was weighed out on cheesecloth. The vegetables were placed into the colander and washed with cool running tap water (3.3 liters min^{-1}) for 30 s. After draining for 1 min, the vegetables were aseptically transferred to a sterile stomacher filter bag. Vegetables were then weighed in the stomacher bag, with an empty stomacher bag used to tare the balance, and 99 ml of Butterfield's phosphate diluent were added. Sample dilution and direct plating for *E. coli* were done as described above. For enrichment of vegetables, 100 ml of double-strength nutrient broth was added to the initial dilution, and the mixture was incubated for 24 h at 35°C. The remainder of the enrichment procedure was the same as described above. The confirmation rates for presumptive *E. coli* isolates obtained following enrichment were 82, 100, and 69% for radish, lettuce, and carrot, respectively, grown in LS, and 93, 92, and 95% for radish, lettuce, and carrot grown in SCL.

Presentation and analysis of data. The log CFU per gram values for all manure-fertilized soil samples taken at a field site (LS or SCL) on a specific date were averaged. Along with the standard error, these values are presented in Figure 3. With the log CFU per gram data and a log–log transformation, the *E. coli* death rate was compared for the two manure-fertilized soils using multiple linear regression *(26).* The proportion of vegetable samples from which *E. coli* was detected was determined for each combination of field site, vegetable type, fertilization-to-planting interval, and fertilization-to-harvest interval (Table 2). Because the number of observations for each combination was fairly small, a Fisher's exact test was performed to compare the different fertilization-to-planting intervals at each site in terms of proportion of samples testing *E. coli* positive; the same comparison was done for the different fertilization-to-harvest intervals at each site *(7).* The function fisher.test in the statistical software R was used to perform this test (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). In particular, for each given vegetable type, an overall test was first performed to determine whether there was any statistically significant difference among all the intervals. If the overall test showed a significant difference (10% significance level), then a pairwise comparison was done between each pair of intervals. This procedure was done for both the fertilization-to-planting intervals and the fertilization-to-harvest intervals.

RESULTS

Prior to manure application (March 2004), *E. coli* was detected on eight of eight LS samples by enrichment and on one of eight samples by direct plating (1.1 log CFU/g). For SCL, all eight samples tested positive for *E. coli* by

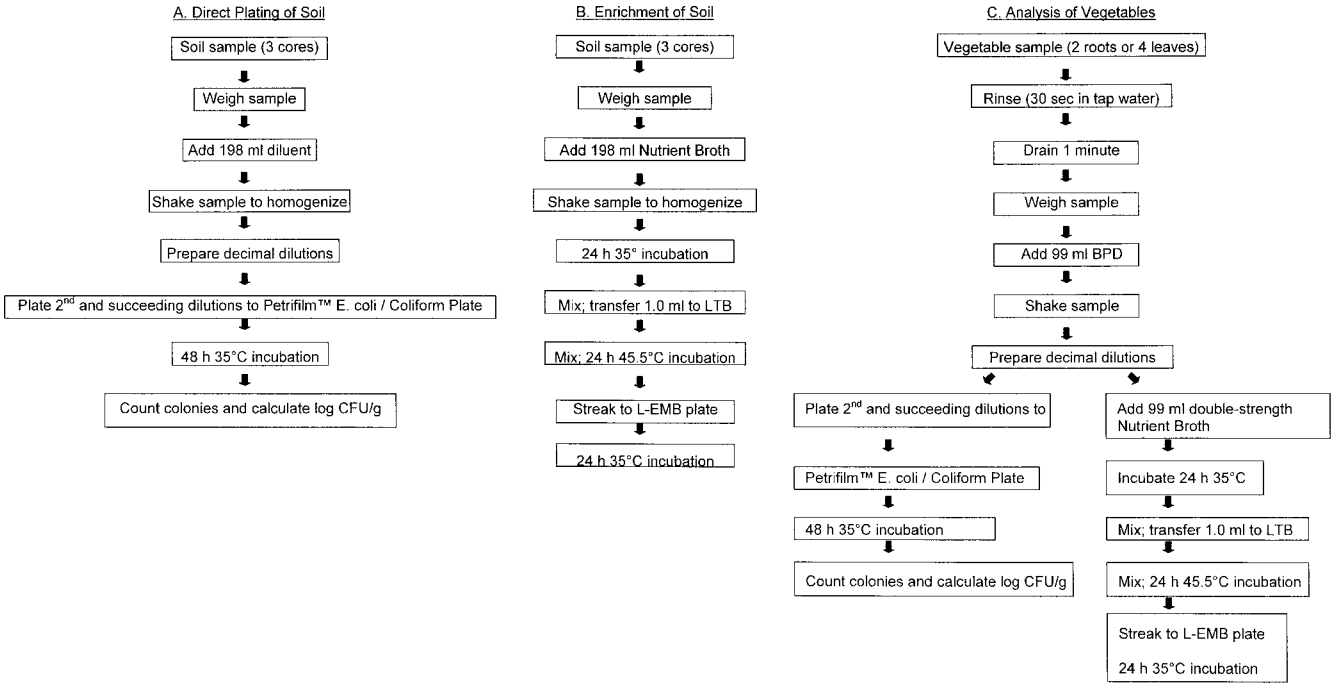

FIGURE 2. *Summary of analysis steps for direct plating of soil (A), enrichment of soil (B), and direct plating and enrichment of harvested vegetables (C).*

enrichment and four samples yielded low numbers (1.2 to 1.6 log CFU/g) of presumptive *E. coli* by direct plating. Following manure application, numbers of *E. coli* cells in the two manure-fertilized soils decreased rapidly from initial levels of about 4.0 log CFU/g to about 1.6 log CFU/g after 7 weeks (Fig. 3). Statistical analysis showed that there was no statistically significant difference between the two soils in the rate of decrease for *E. coli* populations. There was also little difference between *E. coli* levels in soils associated with the three vegetables after planting in a given soil. In LS, levels ranged from <1.3 to 2.3 log CFU/g. In SCL, the ranges were <1.3 to 3.9, 1.3 to 2.9, and <1.3 to 2.4 log CFU/g for soils associated with carrots, lettuce, and radish, respectively. The differences in maximum value for these ranges simply reflect that carrots were planted earliest, followed by lettuce and radishes. At earlier planting times, higher levels of *E. coli* were generally present in the manure-fertilized SCL. Once *E. coli* levels fell to near the detection limit, enrichment analysis detected *E. coli* in all samples of manure-fertilized soil from both sites for the duration of the study (10 additional weeks). As in the 2003 field study, there was evidence of wildlife traffic through the test areas, particularly birds and ground squirrels at West Madison and white-tailed deer at Hancock. This traffic may have contributed to the continued presence of *E. coli* in manure-fertilized soil late in the study. This possibility is supported by the fact that 5 of 11 and 17 of 19 unfertilized LS and SCL control samples, respectively, obtained throughout the study tested positive for *E. coli* after enrichment.

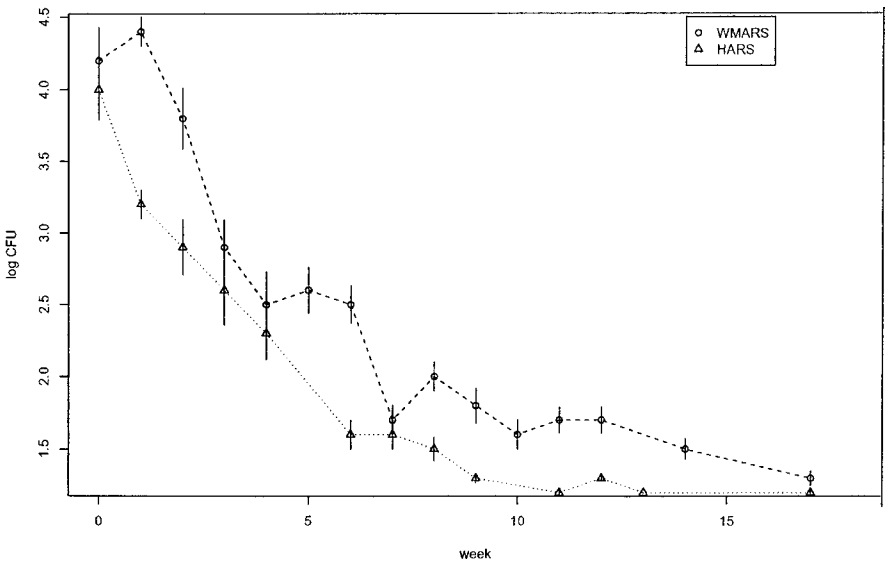

FIGURE 3. *Survival of* Escherichia coli *in noncomposted bovine manure incorporated in loamy sand (Hancock Agricultural Research Station [HARS]) and silty clay loam (West Madison Agricultural Research Station [WMARS]) soils on 13 April and 14 April, respectively. Values are mean with standard error as error bars for 14 to 18 samples. For loamy sand, sample numbers were 14 samples at weeks 2, 3, 9; 15 samples at weeks 0, 1, 4, 6, 7; 16 samples at week 8; 17 samples at week 11; 18 samples at weeks 12, 13, 17. For silty clay loam, sample numbers were 14 samples at weeks 2, 3, 5; 15 samples at weeks 0, 1, 4, 6; 16 samples at weeks 7, 9; 17 samples at weeks 10, 14; 18 samples at weeks 11, 12, 17. Detection limit was 1.3 log CFU/g.*

TABLE 2. *Prevalence of* Escherichia coli *on vegetables grown in loamy sand (LS) and silty clay loam (SCL) soils fertilized with noncomposted bovine manure*[a]

Soil type	Vegetable	Fertilization-to-planting (d)	Fertilization-to-harvest (d)	*E. coli* (+) results
LS	Radish	62	90	3/3
			100	3/3
			111	2/3
			120	3/3
		72	100	0/3
			111	3/3
			120	2/3
		83	111	0/3
			120	1/3
		92	120	0/3
	Lettuce	41	90	1/3
			100	2/3
			111	1/3
			120	0/3
		52	100	1/3
			111	3/3
			120	1/3
		63	111	2/3
			120	0/3
		73	120	0/3
	Carrot	13	90	3/3
			100	1/3
			111	3/3
			120	2/3
		24	100	1/3
			111	3/3
			120	1/3
		34	111	2/3
			120	0/3
		45	120	0/3
SCL	Radish	61	90	3/3
			100	3/3
		72	100	1/3
			110	2/3
		82	110	3/3
			120	1/3
		92	120	2/3
	Lettuce	43	90	2/3
			100	3/3
			110	3/3
			120	1/3
		51	100	2/3
			110	2/3
			120	1/3
		61	110	0/3
			120	1/3
		72	120	0/3
	Carrot	12	90	3/3
			100	0/1
			120	1/1
		22	100	2/3
			110	3/3
			120	2/3
		35	110	3/3
			120	2/3
		42	120	3/3

[a] First number in the results column indicates number of samples yielding positive results following two-step enrichment procedure; second number indicates number of samples tested.

TABLE 3. *Statistical analysis comparing different fertilization-to-planting intervals in terms of prevalence of* Escherichia coli *on harvested vegetables*[a]

Soil type	Radish	Lettuce	Carrot
LS	0.001	0.400	0.086
Significantly different pairs	62 d > 83 d 62 d > 92 d	None	13 d > 45 d
SCL	0.310	0.034	0.99
Significantly different pairs	None	43 d > 61 d 43 d > 72 d	None

[a] Value given is *P* value. Values of 0.10 or lower indicate a significant difference in prevalence among the different intervals. Pairwise comparisons with a statistically significant difference are listed along with < or > to indicate which interval yielded a higher prevalence of *E. coli* (+) samples. Soil types are loamy sand (LS) and silty clay loam (SCL).

No *E. coli* was detected by direct plating of vegetables grown in LS, while 2 of 23 carrot samples (0.6 and 1.0 log CFU/g), 3 of 30 lettuce samples (0.5, 0.6, and 3.0 log CFU/g), and 1 of 21 radish samples (0.8 log CFU/g) from SCL yielded *E. coli* by direct plating. The prevalence of *E. coli* detected by enrichment of vegetables grown in manure-fertilized LS and SCL soils is shown in Table 2. There was clear evidence that increasing the fertilization-to-planting interval in the lower clay LS soil decreased the likelihood of root crop contamination by *E. coli.* Radishes grown in LS consistently tested positive for *E. coli* when the fertilization-to-planting interval was 62 days. Of 12 samples distributed over a 30-day harvest period, only one sample tested negative. In contrast, four of nine samples, five of six samples, and three of three samples with 72-, 83-, and 92-day fertilization-to-planting intervals, respectively, tested negative. For carrots grown in LS, *E. coli*–negative results were obtained for 3 of 12, 4 of 9, 4 of 6, and 3 of 3 samples, with respective fertilization-to-planting intervals of 13, 24, 34, and 45 days, respectively. Statistical analysis of these results showed that the fertilization-to-planting interval significantly influenced *E. coli* prevalence on the two root crops, with some significant pairwise differences (Table 3). The fertilization-to-planting interval had no apparent effect on the prevalence of *E. coli* on lettuce.

The fertilization-to-planting interval apparently did not affect the likelihood of root crop contamination in the higher clay SCL soil, probably because this soil is more likely to adhere to the vegetables. In fact, only 6 of 21 radish samples and 4 of 23 carrot samples overall from SCL tested negative by enrichment for *E. coli.* However, the fertilization-to-planting interval had a significant effect on prevalence of *E. coli* on lettuce grown in manure-fertilized SCL (Table 3). Lettuce with a fertilization-to-planting interval of 43 days yielded *E. coli*–negative results for only 3 of 12 samples, compared with 4 of 9 samples, 5 of 6 samples, and 3 of 3 samples from lettuce with 51-, 61-, and 72-day fertilization-to-planting intervals, respectively.

The fertilization-to-harvest interval appeared to be much less important than the fertilization-to-planting interval in terms of influencing the prevalence of *E. coli* on

TABLE 4. *Statistical analysis comparing different fertilization-to-harvest intervals in terms of prevalence of* Escherichia coli *on harvested vegetables*[a]

Soil type	Radish	Lettuce	Carrot
LS	0.57	0.027	0.005
Significantly different pairs	None	100 d > 120 d 111 d > 120 d	90 d > 120 d 100 d < 111 d 111 d > 120 d
SCL	0.59	0.097	0.23
Significantly different pairs	None	100 d > 120 d	None

[a] Value given is *P* value. Values of 0.10 or lower indicate a significant difference in prevalence among the different intervals. Pairwise comparisons with a statistically significant difference are listed. Soil types are loamy sand (LS) and silty clay loam (SCL).

vegetables grown in manure-fertilized soil. It is clear that fertilization-to-harvest had no significant effect on prevalence of *E. coli* on the fastest growing crop, radishes, from LS (Table 4). There was statistical evidence the fertilization-to-harvest interval in LS affected the likelihood of *E. coli* contamination of the slower growing lettuce and carrot (Table 4). However, this effect may have been at least partially attributable to an abnormally high prevalence of *E. coli* on vegetables harvested 111 days after planting. Of the 27 vegetable samples harvested 111 days after fertilization, 19 were *E. coli* positive (Table 2). In contrast, 8 of 18 samples overall analyzed at 100 days after planting were *E. coli* positive. Of the statistically significant pairwise differences shown in Table 4 for LS, three of five involved the 111-day fertilization-to-harvest interval.

In SCL there was no evidence that fertilization-to-harvest interval affected the likelihood of root crop contamination. The only vegetable for which the fertilization-to-harvest interval had a statistically significant effect was lettuce (Table 4). However, there was also no evidence that using the NOP minimum fertilization-to-harvest interval of 120 days would prevent contamination. For all three types of vegetables harvested from SCL 120 days after fertilization, 14 of 28 samples were *E. coli* positive following enrichment.

DISCUSSION

Other studies have reported on the rate of decrease for manure-borne bacteria in the manure-fertilized soil environment. Baloda et al. *(1)* reported that *Salmonella* Typhimurium DT12 in pig slurry applied to soil was undetectable 21 days after application. In the present study, *E. coli* was easily detected in both manure-fertilized soils during the first month after application, a difference possibly attributable to different initial population densities. Stoddard et al. *(32)* found that the death rate for fecal coliforms in bovine manure-fertilized soil decreased with time. A similar decrease in death rate over time was observed in the present study, with *E. coli* numbers decreasing more slowly after the first month. Van Donsel et al. *(37)* found that fecal coliform levels in manure-fertilized soil decreased by 3 log CFU/g in 2 to 4 weeks, depending on ambient temperature and exposure to direct sunlight. Similar trends were described by Tannock and Smith *(34)* for salmonellae. *E. coli* did not die as rapidly in the present study, perhaps because of the wet conditions in May and June. The rate of decrease in *E. coli* numbers in manure-fertilized soils during 2004 was comparable with that observed in 2003 *(16)* even though considerably more rain fell in 2004. In both years, reintroduction of *E. coli* to plots by wildlife may have played an important role in *E. coli* persistence. Wildlife may be an important vector for pathogenic bacteria in an agricultural setting, particularly if noncomposted manure is piled elsewhere on the farm. It is difficult to control wildlife on a farm, and a realistic evaluation of vegetable contamination must account for wildlife-borne bacteria. Future field studies should attempt to determine the source of *E. coli* isolates, i.e., bovine manure versus wildlife, using genetic fingerprinting techniques. The potential introduction of *E. coli* to agricultural soils by wildlife shows the inherent problems of using a fecal indicator organism that is widespread in domesticated and wild animals. On the one hand, wildlife feces can contain nonpathogenic *E. coli* as well as various pathogenic bacteria *(14, 29–31, 35, 38)*, and evidence suggests that wild birds and rodents can become infected with verocytotoxin-producing *E. coli* from farm animals or vice versa (24). Thus *E. coli* would correctly indicate that fecal contamination of soil had occurred, regardless of the source of feces. On the other hand, nontargeted analysis for *E. coli* would not be definitive if one was trying to evaluate whether noncomposted manure had been properly applied. High levels of *E. coli* in manure-fertilized soil would likely indicate that an insufficient interval had elapsed since fertilization. But low levels of *E. coli* in manure-fertilized soil could either indicate an insufficient interval since fertilization or contamination by wildlife. The fact that indigenous *E. coli* is potentially a very conservative indicator for *E. coli* O157:H7, which is reportedly found at very low levels in bovine manure *(27)*, further complicates the use of *E. coli* testing to evaluate fertilization practices and potential vegetable contamination.

The 2004 results reported here corroborated our 2003 finding that the NOP 120-day minimum fertilization-to-harvest interval will not ensure the absence of fecal bacterial on vegetables grown in Wisconsin soils fertilized with noncomposted bovine manure. As mentioned above, *E. coli* persistence in soil and recontamination of soil via wildlife feces make absolute prevention of contamination unlikely. Our results in the present study also confirmed that decreasing the 120-day fertilization-to-harvest interval to 100 days may slightly increase the risk of contamination under some conditions, e.g., slow-growing crops in sandy soil, lettuce in a higher clay soil. Furthermore, our 2004 results clearly showed that the fertilization-to-planting interval has a greater impact than the fertilization-to-harvest interval on *E. coli* contamination of vegetables grown in sandy soil. These findings are consistent with other work showing that seedling emergence is a critical time for vegetable contamination *(8, 9)*. The high adherence of higher clay soils to veg-

etables, combined with the general in-soil persistence of *E. coli*, results in a high likelihood of *E. coli* contaminating vegetables in higher clay soils, regardless of the fertilization-to-planting interval.

In summary, the results of our 2003 and 2004 field studies do not provide enough support to advocate reducing the NOP minimum fertilization-to-harvest interval from the current 120-day value. Our results do lead to a recommendation for extreme caution in the use of noncomposted bovine manure as a fertilizer in vegetable production. Wisconsin organic vegetable farmers are strongly advised to consider using only composted manure as fertilizer. If composting is not possible, a prudent alternative strategy would be to apply noncomposted bovine manure only in the preceding fall and not at all during the spring and growing season. The risk of contamination of vegetables with manure-borne bacteria cannot be eliminated when noncomposted bovine manure is applied in the spring. If spring fertilization is done, the fertilization-to-planting and fertilization-to-harvest intervals should both be maximized, perhaps by only fertilizing fields used in growing late-season crops.

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