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Short communication

Fecal coliform dispersal by rain splash on slopes

Douglas G. Boyer *

USDA-ARS, Appalachian Farming Systems Research Center, 1224 Airport Road, Beaver, WV 25813, United States

ARTICLE INFO

Article history:
Received 13 September 2007
Received in revised form
2 April 2008
Accepted 3 April 2008

Keywords:
Rain splash
Pathogen dispersal
Land slope

ABSTRACT

The movement of fecal pathogens from land to surface and ground water are of great interest because of the public health implications. Non-structural best management practices that control the timing, volume, and placement of animal manures are commonly used to limit opportunities for fecal pathogens to enter water bodies. Increased infiltration capacity, water and waste diversions, and vegetated filter strips are used to control fecal pathogen movement in surface runoff. Fecal pathogens transported by rain splash could conceivably bypass physical barriers. The relationship between slope angle and the transport of fecal coliform bacteria by rain splash was studied. It was hypothesized that there would be a significant down slope transport of fecal coliform bacteria by raindrops falling on a bare soil surface inoculated with fecal coliform bacteria. Slopes from 0° to 40.8° were studied. The mean splash distance for fecal coliforms was less than 50 mm in all directions at 0° slope and more than 500 mm in the downslope direction on a 40.8° slope. Maximum splash distances ranged from about 400 mm on the horizontal surface to more than 1900 mm in the downslope direction on the 40.8° slope. Sequential downhill movement of fecal coliform (FC) bacteria by repeated rain splash could transport FC directly to water bodies or areas of saturation excess where they will become entrained in overland runoff. Further studies on raindrop and rainfall characteristics, as well as surface cover and soil characteristics, will be necessary to more fully understand the mechanisms of FC transport on sloping land by rain splash.

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1. Introduction

The movement of fecal pathogens from land to surface and ground water are of great interest because of the public health implications (ASM, 1999). Non-structural best management practices that control the timing, volume, and placement of animal manures are commonly used to limit opportunities for fecal pathogens to enter water bodies (NRCS, 1999). Grazing lands in Appalachia tend to be located on sloping landscapes. Sequential downhill movement of fecal coliform bacteria by repeated rain splash could transport fecal coliforms directly to water bodies or areas of saturation excess where they will become entrained in overland runoff. Increased infiltration capacity, water and waste diversions, and vegetated filter strips are used to control fecal pathogen movement in surface runoff (Lim et al., 1998; Tufford and Marshall, 2002). Fecal pathogens transported by rain splash could conceivably bypass physical barriers. Gregory et al. (1959) stated that cells of *Escherichia coli* are readily splashed and that the dispersal of microorganisms by splash is likely to be a highly effective process. Buttleworth and McCartney (1991) found that genetically manipulated bacteria did not splash very far from horizontally oriented leaf surfaces. A survey of the literature failed to find any studies of the dispersal of fecal pathogens by rain splash.

* Tel.: +1 304 256 2833; fax: +1 304 256 2921.
E-mail address: Doug.Boyer@ars.usda.gov.
0168-1923/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.agrformet.2008.04.001

Detachment and dispersal of soil by raindrops is well documented in soil erosion literature (Dunne and Leopold, 1978; Ghadiri and Payne, 1979). Fecal pathogens are transported both as free-floating in the water column as well as adsorbed to particles. Pathogens adsorbed to particles will undoubtedly be transported with soil particles transported by rain splash. The transport of plant pathogens (fungal spores and bacteria) by rain splash is well documented (Fitt and McCartney, 1986; Gregory et al., 1959; Jenkinson and Parry, 1994; Lovell et al., 1999; Madden, 1992; Walklate, 1989; Walklate et al., 1989). Transport of plant pathogens by rain splash might involve simultaneous transport of soil particles depending on whether the plant pathogenic particles are located on soil or plant surfaces (Foster et al., 1985).

The extensive and meticulous studies of fungal spore dispersal by rain splash conducted by Madden and co-workers (Madden, 1992, 1997; Madden and Boudreau, 1997; Madden et al., 1996; Ntahimpera et al., 1997, 1998, 1999; Saint-Jean et al., 2005) have contributed significantly to our understanding of rain splash dynamics in vegetative canopies and row crop agriculture. Those studies found that splash dispersal increased with raindrop size because of increased velocity and kinetic energy (Madden, 1992; Madden et al., 1996; Ntahimpera et al., 1997). The relationships of spore dispersal to rainfall intensity and storm duration were complicated partially because of wash off from plant surfaces and soil infiltration (Madden, 1992, 1997; Madden et al., 1996). Increasing surface roughness and plant cover leaf area index reduced the amount of spore dispersal by rain splash (Madden, 1992; Madden and Boudreau, 1997; Ntahimpera et al., 1998).

Dunne and Leopold (1978) stated that rain splash on slopes results in net downhill movement of soil particles. The importance of rain splash in the erosion of soil has been attributed to the early phase of soil erosion, or detachment, by several investigators (Smith and Wischmeier, 1962). The mechanics of rain splash depend upon the raindrop kinetic energy at the point of impact. The kinetic energy is related to factors such as raindrop size, angle of inclination, and fall velocity (Madden, 1992; Park et al., 1983; Pietravalle et al., 2001; Stedman, 1979; Walklate, 1989; Walklate et al., 1989). Depth of standing water and impact surface properties at the impact site are important (Allen, 1988; Finney, 1984; Huber et al., 1997) for determining splash dispersal characteristics. Wind velocity and direction also strongly affect splash dispersal patterns (Erpul et al., 2004).

The relationship between ground slope angle and the transport of fecal coliform bacteria by rain splash was studied. It was hypothesized that the number of rain-splashed fecal coliform bacteria and mean distance of dispersal downslope would increase relative to upslope dispersal as ground slope increases. A short, controlled experiment of rain splash dispersal of fecal coliform bacteria from bare soil on various slopes was used to investigate the hypothesized relationship.

2. Materials and methods

2.1. Mechanistic model

Zaslavsky and Sinai (1981) developed a mechanistic theory of down slope flow resulting from rain splash. They experimentally demonstrated that downslope horizontal flow of splashes was proportional to the slope. Their basic equations of horizontal flight distance of splash droplets in the downslope (x_d) and upslope (x_u) directions for angle of slope α were presented as

$$x_d = \frac{2}{g} V_o^2 \cos^2 \beta (\tan \beta + \tan \alpha) \quad (1)$$

$$x_u = \frac{2}{g} V_o^2 \cos^2 \beta (\tan \beta - \tan \alpha) \quad (2)$$

where g is gravitational acceleration, V_o is the initial velocity of outgoing splash droplets, and β is the angle between the flight of the outgoing splash droplet and the horizon. Typical values in the literature for V_o range between 1 and 5 m s^{-1} and β typically ranges between 20° and 60° on fine soils (Allen, 1988; MacDonald and McCartney, 1987; Saint-Jean et al., 2004). They found that the assumption of a uniform β around the drop impact could be made. Eqs. (1) and (2) are identical except for the sign before the tangent of the slope angle. The multiplier ($\tan \beta \pm \tan \alpha$) increases with increasing slope angle in the downslope direction and decreases with increasing slope angle in the upslope direction.

2.2. Measurements

Water samples from a limestone spring previously shown to contain high concentrations of fecal coliform bacteria of agricultural origin (Pasquarell and Boyer, 1995) were used to provide a source of fecal coliform bacteria (FC) for this study. Presumptive FC colonies from membrane filtration of the spring water samples were harvested from 55 ml Petri plates containing standard m-FC agar (Membrane Faecal Coliform Agar, Oxoid, Hampshire, England) prepared according to standard methods (Eaton et al., 1995). The harvested colonies were transferred to tubes containing standard Bacto Lactose Broth (Difco) bacterial growth and enrichment media. The tubes were incubated at 37° C to produce a high population density source of FC. The FC was not identified to genus and species. Fecal coliforms are facultatively anaerobic, rod-shaped, gram-negative, non spore-forming bacteria that ferment lactose and include the fecal-origin genera; *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. *E. coli* is usually the predominant genus and enrichment in lactose broth is known to yield maximum growth of *E. coli* and *Enterobacter*. Recent tests of the source spring water (D. Boyer, unpublished data) show that *E. coli* make up more than 90% of the FC colony forming units (CFU).

Experiments were carried out on uniformly sloping (α) surfaces of 0°, 4.8°, 10°, 14.6°, 19°, 23.1°, 26.6°, 35.3°, and 40.8° from horizontal. The sloping surface was constructed of plywood supported on one end at heights above the floor which resulted in the experimental slope angles. Polystyrene m-FC agar Petri plates, 55 mm diameter × 14 mm depth, were lined up edge to edge up and down the slope. Another line of Petri plates was arranged perpendicular to the slope-oriented line (Fig. 1). Two other lines of Petri plates were arranged midway between the first two lines. The four lines of Petri

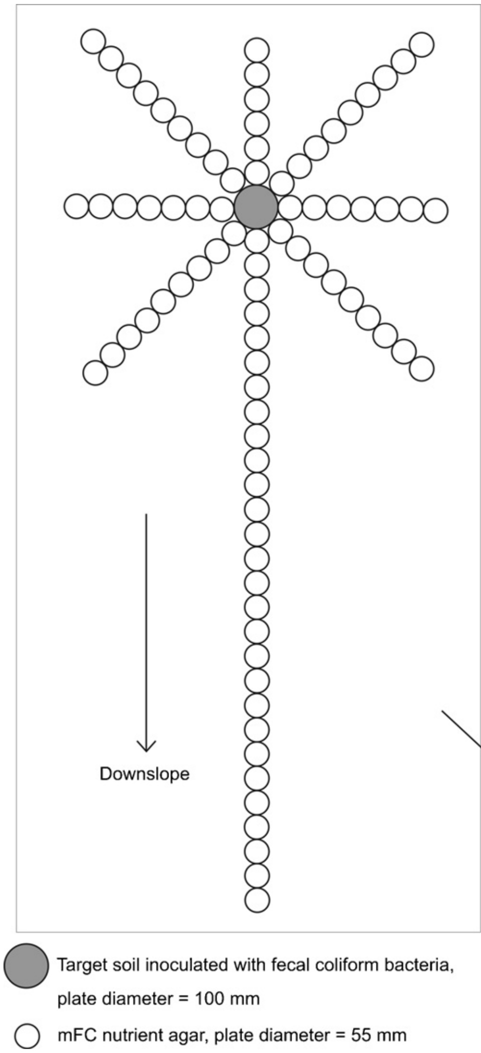

Fig. 1 – Layout of center soil plate and mFC nutrient agar plates for the 19° slope experiment.

plates intersected at a common point so that eight lines of plates radiated out azimuthally at 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° with 0° being the upslope direction and 180° the downslope direction. All of the m-FC agar Petri plates except the one at the intersecting point contained m-FC nutrient agar filled to a depth of about 7 mm.

The intersection plate (100 mm diameter × 15 mm depth) contained about 7 mm of sterilized soil spiked with FC bacteria. The soil was sterilized by flowing steam in an autoclave prior to spiking. The soil plate was prepared by adding 50 g soil (dried, ground, and sieved at 2 mm) and 20 ml deionized, distilled water to simulate 'field capacity' conditions. The original soil was obtained from the Ap horizon of the Frankstown Silt Loam (Fine-loamy, mixed, semiactive, mesic Typic Hapludults) in Greenbrier County, West Virginia. The soil was inoculated with 10^8 FC by hand spraying a mist of 5 ml of water containing the inoculum directly on the soil surface. FC density of the inoculant solution was determined by optical density spectrometry (Thermo Spectronic, Madison, WI). Immediately after inoculation the soil plate was placed on the experimental sloping surface at the center of the m-FC agar Petri plates (Fig. 1). The rain simulation started immediately to minimize time of plate exposures to the atmosphere.

Preliminary splash experiments using a red dye (Acid Red 1; CAS No. 3734-67-6) and Kraft paper provided information for adjustment of line lengths downslope, upslope, and sideways from the intersection of Petri plates as the experimental slopes changed. Two additional m-FC nutrient agar plates served as controls during each splash experiment. One control was left covered in the laboratory media storage refrigerator and the other control was uncovered in the splash experiment room during each experiment. The uncovered control plates were kept out of range of splash droplets.

Rainfall simulation was performed by dripping by hand 100 drops of deionized, distilled water from a plastic transfer pipette held 2 m above the target plate. The pipette produced 20 drops ml^{-1} so 5 ml of water dropped onto the target plate. Assuming that the drops were spherical, the average drop diameter was 5.56 mm. Hand movements while creating the drops caused the drops to strike randomly across most of the plate area.

At the conclusion of each rainfall simulation all of the mFC nutrient agar plates (including the controls) were quickly covered and sealed in Kapak SealPAK^{TM} pouches. The plates were then incubated in a water bath at 44.5° C. FC colonies were enumerated by counting blue colonies on the m-FC agar plates following 22–24 h of incubation. Plate counts were recorded as CFU $plate^{-1}$.

3. Results

The simulated rain of 5 ml for each test represented a total rainfall of 6.4 mm. Further rainfall on the center target plates would have created pooling since the plates were non-draining. All of the control plates tested negative for the presence of FC.

The distribution of bacteria splashed was symmetrical in all directions from the center target plate at the lower slope angles of 0° and 4.8°. There was a shift of less FC splashing upslope and more FC splashing downslope at slope angles of 10° and greater. Very few FC splashed upslope at the two steepest slope angles.

The changes in distribution of FC upslope and downslope of the target plate at different slope angles are illustrated by Fig. 2. At the lower slope angles the distributions tend to be symmetrical and concentrated close to the center target plate. As slope angles increase the distributions are skewed to the right indicating a greater proportion of the FC splashing further down the slope. Fig. 2 shows relative FC (FC_R) counts calculated as

$$FC_R = \frac{FC_{xn}}{FC_{d1}} \quad (3)$$

where n is the plate number from the center target plate in the downslope ($x = d$) or upslope ($x = u$) direction. This normalization scheme sets the relative FC counts in the first downslope plate of each of the slope angle simulations as unity. FC

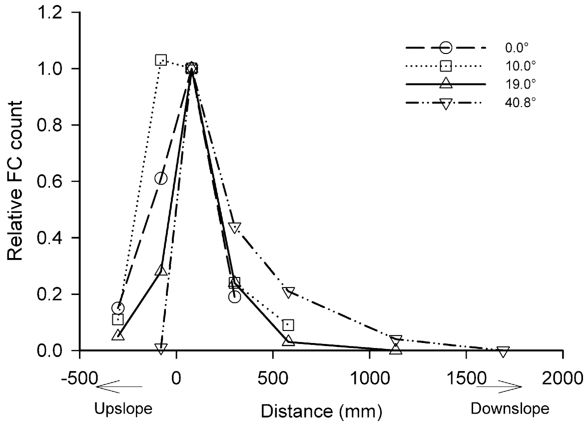

Fig. 2 – FC counts relative to the FC count in the first downslope plate versus distance upslope and downslope at four slope angles.

colonies were found in plates more than 1.5 m from the target plate in the two steepest slope angles and more than one meter from the target plate at all slope angles of 10° and steeper.

The average downhill shift of the center of gravity of the splashed FC was determined as the weighted mean distance traveled by the FC, or the sum of the products of FC counts and distances at a slope angle divided by the sum of FC counts at that slope angle. Distance was assumed positive in the downhill direction and negative in the uphill direction. Since the entire distribution of FC in the uphill direction might not have been accounted for the downhill shift was calculated both with uphill distances included and with uphill distances excluded. Fig. 3 shows the downhill shifts of the center of gravity, of the splashed FC versus slope angle. Since the experiment was not replicated at each of the slope angles, error statistics could not be included. As the slope angle increases inclusion of the uphill data is less important because so few FC splash any significant distance in the uphill direction. Observed centers of gravity for both calculation methods (i.e., inclusion or exclusion of uphill splash data) are virtually identical for slope angles above 30°.

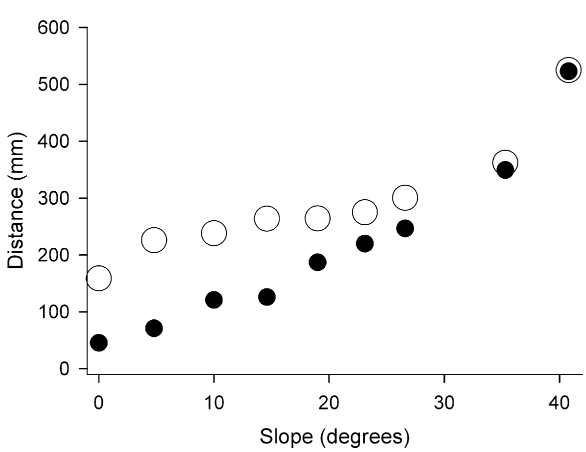

Fig. 3 – Observed weighted mean downslope distance of rain splash transport of observed FC counts when uphill splash data were excluded (open circles) and included (solid circles) in the weighted mean.

4. Discussion and conclusions

The direct relationship between slope and movement of FC by rain splash was expected and confirmed by this study. Simulated raindrop size of 5.56 mm was large and probably represents median drop diameters of high-intensity storms in the eastern United States (Laws and Parsons, 1943; Park et al., 1983). A 5.5 mm drop has a terminal velocity of about 8.7 m s^{-1} (Madden, 1992) and the study drops would have reached a velocity of about 7 m s^{-1} as determined from equations published by Park et al. (1983). As raindrops fall through the atmosphere they quickly reach terminal velocity and break up into smaller drops. Since this experiment was only concerned with slope affect on patterns of rain splash dispersal of FC bacteria the raindrop size was not a subject of study. Further studies of FC bacteria dispersal on slopes should consider raindrop size distributions, raindrop velocity at impact, and kinetic energy. Madden (1992, 1997) found that rain drop size was the single most important factor determining splash dispersal of fungal spores because of the direct relationship between drop size with terminal velocity and kinetic energy. Yang et al. (1991) studied the effect of leaf angles in strawberry canopies and found significant dispersal effects related to leaf slope angle. They found that mean splash angle was dependent on both impact velocity and impact angle.

Several factors besides raindrop and rainfall characteristics will be expected to influence the launching and dispersal of FC bacteria by raindrop splash. The density of FC bacteria at the soil surface is important. Since the splashed droplets have a maximum carrying capacity for bacterial cells there will be a surface density of FC bacteria above which there is no increase in the number of bacteria cells transported. There is also the possibility that the surface density of FC bacteria will be diminished as cells are splashed away or infiltrated into the soil (Madden, 1992). However, FC bacteria transported in splash from uphill sites will replenish at least a portion of those splashed away.

Surface cover will probably have the greatest impact on FC bacteria transport by rain splash. Interception of raindrops by plant canopies will diminish their kinetic energy thus reducing the amount of rain splash and dissemination of splash droplets (Madden, 1997; Yang and Madden, 1993). Plant cover also serves as a barrier to outgoing rain splash droplets (Pietravalle et al., 2001; Saint-Jean et al., 2004). Dissemination of plant pathogens within plant canopies by rain splash has been shown to be an important transport process (Jenkinson and Parry, 1994; Lovell et al., 1999; Walklate, 1989; Walklate et al., 1989) because of 'stair stepping' by repeated splashing. Similar mechanisms of dispersal for fecal pathogens by repeated splashing might be important; especially as surface slope angles increase. Buttleworth and McCartney (1991) found that genetically manipulated bacteria did not splash far laterally from horizontally oriented leaves and most of the bacteria were washed off to the soil. Furthermore, redistribution of FC pathogens into the upper portions of forage canopies might be an important potential vector of microorganism transmission to grazing animals. In a study of splash dispersal of fungal spores in wheat Paul et al. (2004) found that spores were routinely splashed to upper parts of the canopy and rain splash played an important role in the spread of Fusarium head blight.

Transport of FC bacteria by rain splash from unobstructed soil surfaces was confirmed and significant. Increasing slope angles increased the proportion of bacteria splashed in the downhill direction. Virtually no FC bacteria were transported upslope by rain splash at the steepest slope angles studied. Grazing lands in Appalachia tend to be located on sloping landscapes. Models of pathogen transport need to consider rain splash effects on sloping landscapes if a full accountability of transport mechanisms are to be made. Further studies on raindrop and rainfall characteristics, as well as surface cover and soil characteristics, will be necessary to more fully understand the mechanisms of FC transport by rain splash on slopes.

Acknowledgements

Contribution of the USDA – Agricultural Research Service. Trade and company names are used for the benefit of readers and do not imply endorsement by the USDA. This research was supported by the 205 Rangeland, Pasture, and Forages National Program of the USDA-ARS. The authors gratefully acknowledge the technical assistance of Elsa Cook, Derek Hall, and Levi Burns.

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