

# Efficacy of Vegetated Buffer Strips for Retaining *Cryptosporidium parvum*

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## ABSTRACT

Overland and shallow subsurface hydrologic transport of pathogenic *Cryptosporidium parvum* oocysts from cattle feces into surface drinking water supplies is a major concern on annual grasslands in California's central and southern Sierra Nevada foothills. Soil boxes (0.5 m wide  $\times$  1.1 m long  $\times$  0.3 m deep) were used to evaluate the ability of grass vegetated buffer strips to retain  $2 \times 10^8$  spiked *C. parvum* oocysts in 200-g fecal deposits during simulated rainfall intensities of 30 to 47.5 mm/h over 2 h. Buffers were comprised of Ahwahnee sandy loam (coarse-loamy, mixed, active, thermic Mollic Haploxeralfs; 78:18:4 sand to silt to clay ratio; dry bulk density = 1.4 g/cm<sup>3</sup>) set at 5 to 20% land slope, and  $\geq 95\%$  grass cover (grass stubble height = 10 cm; biomass = 900 kg/ha dry weight). Total number of oocysts discharged from each soil box (combined overland and subsurface flow) during the 120-min simulation ranged from  $1.5 \times 10^6$  to  $23.9 \times 10^6$  oocysts. Observed overall mean log<sub>10</sub> reduction of total *C. parvum* flux per meter of vegetated buffer was 1.44, 1.19, and 1.18 for buffers at 5, 12, and 20% land slope, respectively. Rainfall application rate (mm/h) was strongly associated with oocyst flux from these vegetated buffers, resulting in a decrease of 2 to 4% in the log<sub>10</sub> reduction per meter buffer for every additional mm/h applied to the soil box. These results support the use of strategically placed vegetated buffers as one of several management strategies that can reduce the risk of waterborne *C. parvum* attributable to extensive cattle grazing on annual grassland watersheds.

**C**RYPTOSPORIDIUM PARVUM is a ubiquitous protozoal parasite, with specific genotypes able to be transmitted between domestic animals and humans (Peng et al., 1997; Spano et al., 1997; Awad-El-Kariem et al., 1998; Okhuysen et al., 1999; Xiao et al., 2002). Vegetated buffers are widely advocated as a management practice to minimize the likelihood that animal agricultural operations contaminate surface water with enteric microorganisms prevalent in fecal matter, such as generic *E. coli* (Young et al., 1980; Coyne et al., 1995; Younos et al., 1998; Entry et al., 2000; Rosen et al., 2000). Considerably less work has been conducted on the ability of vegetated buffers to remove pathogenic microorganisms from overland and shallow subsurface flow, such as the protozoa, *C. parvum* (Mawdsley et al., 1996a; Tate et al., 2000; Atwill et al., 2002; Trask et al., 2004). It can be hypothesized that the efficiency of buffer strip filtration for *C. parvum* will be considerably greater than the efficiencies observed for enteric bacteria (Gifford and Hawkins, 1978, Gee and Bauder, 1979), given the fact that enteric bacteria such as *Salmonella* and *E. coli* are

smaller rod-shaped pathogens ranging in length from 2 to 5  $\mu$ m and 0.5 to 1.5  $\mu$ m in width, while the infectious stage of *C. parvum* (oocyst) is spherical and has a diameter of 5 to 6  $\mu$ m. It has been suggested that the primary mechanism by which vegetated buffers remove waterborne microbial pathogens is via infiltration of overland flow into the soil profile, followed by subsurface filtration and adsorption (Harter et al., 2000; Atwill et al., 2002; Trask et al., 2004). Larger diameter pathogens such as *C. parvum* are likely to be more susceptible to filtration compared to the smaller enteric bacteria. The situation is less clear for adsorption given the physicochemical processes governing attachment of a biological colloid onto a solid substrate (Walker and Montemagno, 1999) and the ability of *C. parvum* to desorb from sediments in a time-dependent manner (Harter et al., 2000).

When enteric pathogens are excreted by a host in a matrix of fecal material onto the terrestrial component of a watershed, at least three events need to occur in order for *C. parvum* to reach surface water to become a waterborne hazard. First, *C. parvum* oocysts need to be released from the fecal matrix; second, the entrained oocysts need to avoid such processes as filtration, adsorption, and settling while being transported in overland and subsurface flow to a receiving body of water (e.g., river or lake); and third, oocysts need to remain infective during the completion of the first two processes. We have determined previously that a meter of vegetated buffer can function to remove 1 to 3 log<sub>10</sub> of waterborne *C. parvum* oocysts entrained in overland flow (Atwill et al., 2002). The objective of this project was to assess the log<sub>10</sub> reduction per meter of vegetated buffer when *C. parvum* oocysts are instead placed into a fecal matrix, as occurs naturally on watersheds with vertebrate hosts, and to determine if land slope (%) influences the ability of a buffer to retain oocysts. For this experiment, we simulated the vegetation, soil, land slope, and rainfall conditions of annual grasslands found in California's central and southern Sierra Nevada foothills. These grasslands occupy over 1.8 million ha in California and are a critical source of the state's surface drinking water supply. Approximately two-thirds of the state's drinking water reservoirs are located within annual grassland landscapes (Forest and Rangeland Resources Assessment Program, 1988).

## MATERIALS AND METHODS

### Overall Study Design

The experimental unit was a soil box designed to allow collection of overland and subsurface flow. Twelve soil boxes were filled with soil and planted with annual grass typical of south Sierra Nevada foothill rangelands. Three land slope treatments (5, 12, and 20%) were tested across four trials (reps). Bovine feces spiked with *C. parvum* oocysts was placed 1.0 m up-slope from the bottom of the soil box and rainfall

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was applied to the box via one of three stationary nozzle rainfall simulators for 120 min. Thus, during each trial three rainfall simulators (A, B, and C) individually and simultaneously applied rainfall to three soil boxes, each box set to one of the land slope treatments. Overland and subsurface flow volume from each box was measured and subsampled for oocyst concentration throughout the 120-min rainfall-runoff trial.

### Soil Box Design

Twelve soil boxes were constructed of 1.9-cm plywood and sealed against leaks and moisture. Dimensions were 0.5 m wide  $\times$  1.1 m long  $\times$  0.3 m deep, allowing us to determine the filtration or retention efficiency of a meter of vegetated buffer while allowing for reasonable root development of planted vegetation. Boxes were designed to capture overland flow after it had traversed the vegetated buffer, while subsurface flow was captured through a slot at the bottom of the seepage face. The junction between the soil surface and the inner side of the box wall was sealed with a strip of waterproof sealant to minimize seepage of surface flow along the vertical side of the box. The slope of each box was adjusted individually to achieve the desired land slope treatments.

### Simulated Watershed Scenario

#### Landscape

Herbaceous vegetation in southern Sierra Nevada foothills is composed of annual grasses and forbs such as annual ryegrass (*Lolium multiflorum* Lam.), wild oats (*Avena fatua* L.), soft chess (*Bromus mollis* L.), and redstem filaree [*Erodium cicutarium* (L.) L'Her] found either as open grasslands or as understory for scattered blue oak (*Quercus douglasii* Hook and Arn.), interior live oak (*Quercus wislizenii* A.D.C.), or foothill pine (*Pinus sabiniana* Douglas) (Griffin, 1977). Soils are sandy loams derived from decomposing granite parent materials with soil depths typically  $\leq 70$  cm. Topography on these rangelands is rolling to steep uplands (8–35% land slope) with significant swale areas (3–8% land slope) formed from alluvial deposits. Swales are intermittent wetlands which become saturated with lateral subsurface and saturated overland flow from surrounding uplands, serving as variable source areas for stream flow generation (Hewlett and Hibbert, 1967; Bernier, 1985). Precipitation in these rangelands falls almost entirely as rainfall from October through April.

#### Vegetation Type

Annual ryegrass was selected for use in this experiment to simulate soil surface cover typical of open grasslands and understory in these rangelands. We used 150 to 200 g of seed per box, which resulted in  $\geq 95\%$  soil surface cover. Soil boxes were planted with annual ryegrass in October and allowed to mature over the winter growing season, with the experiment conducted the following May. To simulate moderately grazed conditions (900 kg grass as dry weight/ha), the annual ryegrass was clipped to 10 cm one day before the application of feces and simulated rainfall. Grass roots extended the entire 0.3-m depth of the soil boxes. Herbaceous vegetation root zone depth on annual rangelands is approximately 0.2 to 0.4 m

#### Soil Type

The Ahwahnee coarse sandy loam soil series was selected for use in this experiment as a rangeland soil found extensively

throughout this region (Ulrich and Stromberg, 1990). Soil from the A horizon (0–30 cm) of a typical Ahwahnee soil profile was collected from an open grassland site in Madera County, CA. Soil was thoroughly mixed and soil boxes filled. Soil in the boxes was compacted to a mean bulk density of 1.40 g/cm<sup>3</sup> (SD = 0.07 g/cm<sup>3</sup>) to simulate soil compaction conditions typical of heavy grazing in the region (Tate et al., 2004). Mean values for other important soil properties were pH = 6.5; cation exchange capacity = 6.7 cmol/kg; organic matter = 1.1%; texture = 78.3% sand, 17.7% silt, 4.0% clay; and moisture retention capacity = 10.8% at 0.03 MPa, 7.7% at 0.1 MPa, 4.8% at 0.5 MPa, 4.6% at 1 MPa, and 4.6% at 1.5 MPa.

### Land Slope Treatment

Land slope treatment of 5% was chosen for this experiment to simulate swale or variable source area conditions for the southern Sierra Nevada and a 12 and 20% land slope treatment was chosen to simulate upland or hill-slope conditions for this region of California. A further justification for choosing 5, 12, and 20% land slope treatments was that these slope classes receive significantly different amounts of beef cattle fecal deposition and thus potential oocyst loading rates for grazed rangeland in the southern Sierra Nevada (Tate et al., 2003). For example, we have found that cattle fecal loading rates decrease 50 to 99% at grazed locations with a 20 to 30% land slope compared to sites with a 1 to 5% land slope.

### Rainfall Simulation Trial

For each trial ( $n = 4$ ), rainfall was emitted from three stationary nozzle emitters (A, B, C), adapted from Wilcox et al. (1986). Each rainfall emitter was positioned 2 m above the surface of the soil box, inside a small shed to minimize lateral drift. In this configuration the emitters generated a circular rainfall pattern with a diameter of approximately 1.1 m at the soil box surface. Thus, the rate of water emission did not equal the rate of water application to the surface of each soil box. Soil boxes were consistently placed so that the soil box center was exactly under the rainfall emitter. Variable amounts of simulated rainfall were actually received by each soil box due to variability in the pattern of water emission between rainfall emitters and some drift of small water drops. Each emitter was calibrated to produce approximately 53 mm/h of rainfall, measured directly underneath the emitter as the total volume produced in a 5-min period at the start and finish of each rainfall simulation. We applied water to the soil boxes at this rate for 2 h, simulating a storm event for the south Sierra Nevada foothills with a significantly greater than a 100-yr return interval (Hershfield, 1961), a worst case precipitation runoff event. Our previous work on this soil type indicates that due to inherently high infiltration capacity, extreme rainfall events and saturated antecedent soil moisture conditions are required to exceed infiltration capacity and generate overland flow (Tate et al., 2000, 2004). Variability between simulators and trials generated a realized mean (SD), minimum, and maximum water emission rate of 53.0 (1.5), 50.9, and 55.7 mm/h across all 12 application events (four trials  $\times$  three emitters per trial), respectively. Measured rainfall application rates per soil box ranged from 30 to 47.5 mm/h, or from approximately a 50- to 100-yr return period, 2-h duration storm amount (Hershfield, 1961). This variation in rainfall application rate was successfully addressed and capitalized on as a covariate in statistical analysis as described below.

Each land slope treatment was present in each trial, and the position of each land slope treatment was rotated among simulators (A, B, and C) between trials. For each trial, all

three soil boxes were irrigated for 2 h immediately prior fecal deposit application to create saturated soil conditions, and thus simulate worst case antecedent soil moisture conditions for oocyst transport during storm events. After the simulators were calibrated and boxes saturated, a 200-g deposit of bovine fecal material spiked with  $1 \times 10^6$  *C. parvum* oocysts/g was placed 1.0 m up from the bottom of the box and on top of the 10 cm annual ryegrass stubble and rainfall simulation continued for 2 h. Thus, the total oocyst spike per soil box was  $2 \times 10^8$ . This concentration of oocysts was designed to represent a highly infected calf or a very worst-case scenario for fecal shedding by California beef cattle, given that oocyst concentrations shed by adult beef cattle on California rangeland are substantially less (Hoar et al., 2000; Atwill et al., 2003). Overland and subsurface flow was collected separately as 5-min composite samples, with the total volume measured for each sample. For each flow path, composite samples from time intervals at -5 to 0, 0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 35 to 40, 45 to 50, 55 to 60, 75 to 80, 95 to 100, and 115 to 120 min were analyzed for *C. parvum* oocysts.

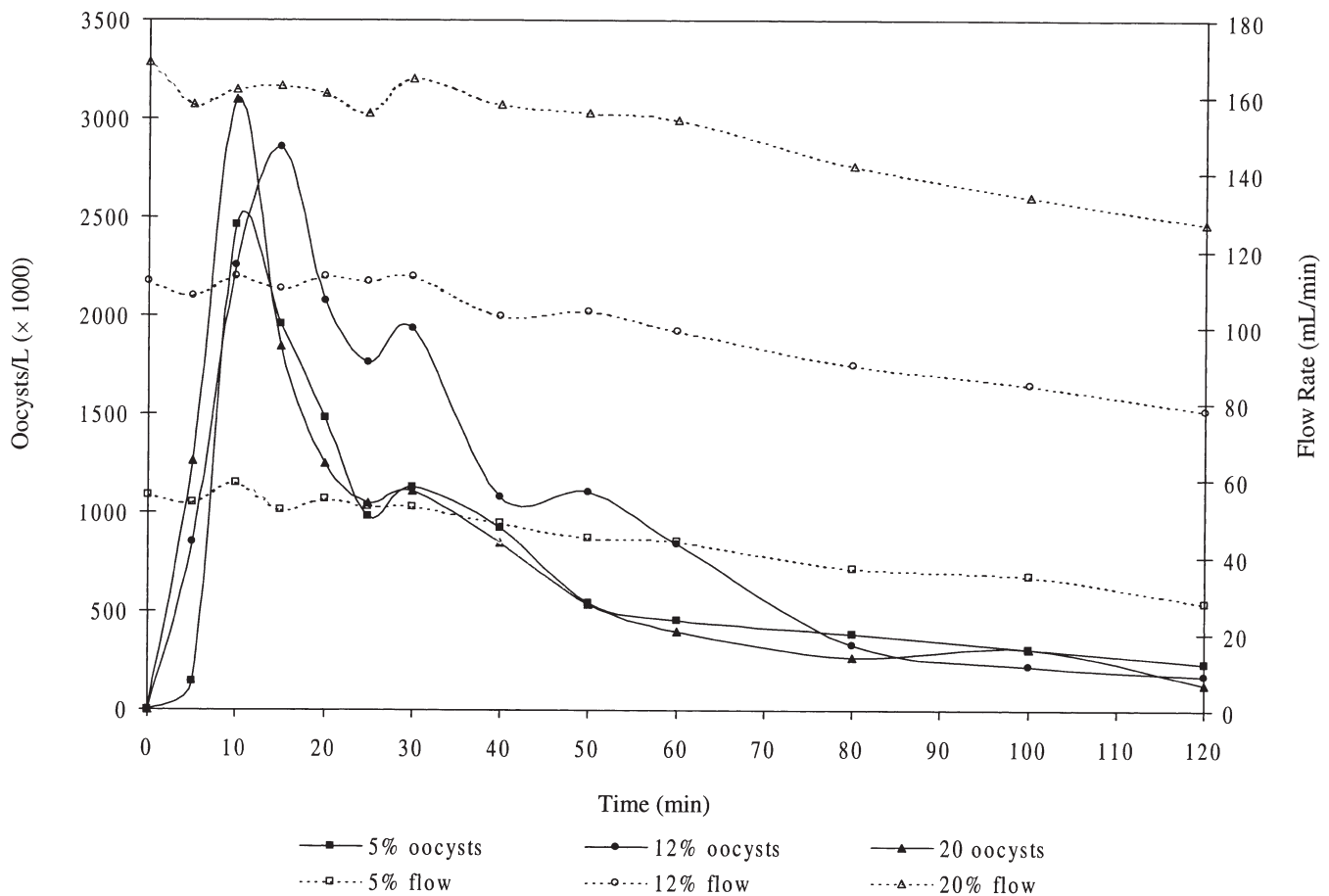
### Source and Purification of *Cryptosporidium parvum* Oocysts

Naturally infected dairy calves from two local commercial dairies were the source of wild-type *C. parvum* oocysts. We have determined previously that these oocysts are classified as bovine genotype A, using the genotyping scheme described

by Xiao et al. (1999). Using an acid fast procedure to detect oocysts (Harp et al., 1996), samples having more than 25 oocysts per  $\times 400$  microscopic field were washed through a series of 40, 100, 200, and 270 mesh sieves. The resulting suspension was decanted off and centrifuged at  $1000 \times g$  for 10 min. Supernatant was discarded and the pellet washed in Tween water (0.01% Tween 80 in deionized water, v/v). Discontinuous sucrose gradient was used to purify *C. parvum* oocysts from fecal suspensions (Arrowood and Sterling, 1987). The concentration of purified oocysts was determined as the arithmetic mean of six separate counts using a phase contrast hemacytometer. Approximately  $2.4 \times 10^9$  oocysts were added to 2400 g of fresh beef cattle fecal material negative for *C. parvum*.

### Quantification of *Cryptosporidium parvum* Oocyst Concentration in Overland and Subsurface Flow

Quantitative immunofluorescent microscopy, adjusted for percent recovery, was used to enumerate *C. parvum* oocysts in water samples (Harter et al., 2000; Atwill et al., 2002). One hundred microliters of 10% Tween 80 and 10% SDS were added to 50-mL aliquots of overland flow and mixed 5 min on a hand wrist shaker. Suspension was centrifuged at  $1000 \times g$  for 15 min and supernatant removed until 1 mL of residual pellet and suspension remained. One hundred microliters of 100% formalin was added and suspension transferred to a 1.5-mL microcentrifuge tube, centrifuged at  $11\,600 \times g$  for 5 min, and supernatant removed until 1 mL of residual pellet



**Fig. 1.** Mean *Cryptosporidium parvum* oocyst concentration breakthrough curves in overland flow from 0.5-m-wide  $\times$  1.1-m-long  $\times$  0.3-m-deep soil boxes set to three land slope treatments, planted with grass, filled with a sandy loam soil, spiked with 200 g of cattle fecal deposit with  $2 \times 10^8$  oocysts, and subject to simulated rainfall intensities of 30 to 47.5 mm/h for 2 h.

and supernatant remained. Ten microliters of suspension were enumerated for *C. parvum* oocysts by fluorescence microscopy using commercially prepared well slides and a fluorescein isothiocyanate-labeled-anti-*Cryptosporidium* antibody (Meridian Diagnostics, Cincinnati, OH). For subsurface samples, we used a similar procedure, except that 100- to 250-mL aliquots were concentrated down to 100 to 500  $\mu\text{L}$ . Positive and negative controls were included with each run.

To determine percent recovery for immunofluorescent microscopy, we collected a total of eight overland and subsurface negative control water samples and added purified *C. parvum* oocysts to a final concentration of 1000 and 40 oocysts/mL, respectively. The immunofluorescent microscopy procedure was then performed as described above.

### Total Oocyst Discharge Calculation and Statistical Analysis

The total number of oocysts that discharged from each soil box during each trial and adjusted for the percent recovery of the assay (outcome variable) was calculated from our set of twenty-six, 5-min composite samples collected from each soil box for overland ( $n = 13$  composite samples) and subsurface flow ( $n = 13$  composite samples). Equation [1] was used to calculate overland and subsurface oocyst discharge in each composite sample, such that the number of observed oocysts per unit volume is the total number of *C. parvum* oocysts estimated via immunofluorescent microscopy for each composite sample, the total volume per time period is the total

volume of water collected over the 5-min sample period, and the percent recovery for the immunofluorescent microscopy procedure was estimated as described below:

$$\frac{\text{total oocysts}}{\text{composite sample}} = \quad [1]$$

$$\frac{(\text{number of observed oocysts/unit volume composite sample}) (\text{total volume}/5\text{-min sample period})}{(\% \text{ recovery})}$$

These 13 values (total oocysts/composite sample) were then used to generate a soil box specific profile of the 120 min breakthrough curve, or instantaneous flux, of oocysts discharged from the soil box as overland and subsurface flow. The area under this curve was estimated by fitting a cubic spline to these 13 data points through time and solving the integral from time = 0 to 120 min (Stata Corporation, 2001). Total number of oocysts discharged per plot was then finally calculated as the sum of the area under the overland flow and subsurface flow breakthrough curves for each box. These values for the total number of oocysts discharged from each soil box were used to generate estimates of the mean  $\log_{10}$  reduction per meter of buffer based on an initial load of  $2 \times 10^8$  *C. parvum* oocysts. Mean  $\log_{10}$  reduction was calculated as:  $\log_{10}(\text{initial load of oocysts deposited on the soil box}) - \log_{10}(\text{combined number of oocysts discharged from the soil box as overland and subsurface flow over 120 min})$ .  $\log_{10}$  reduction is a standardized parameter for conveying the performance of a treatment process that removes waterborne contaminants such as *C. parvum* from raw water used for municipal or other such purposes.

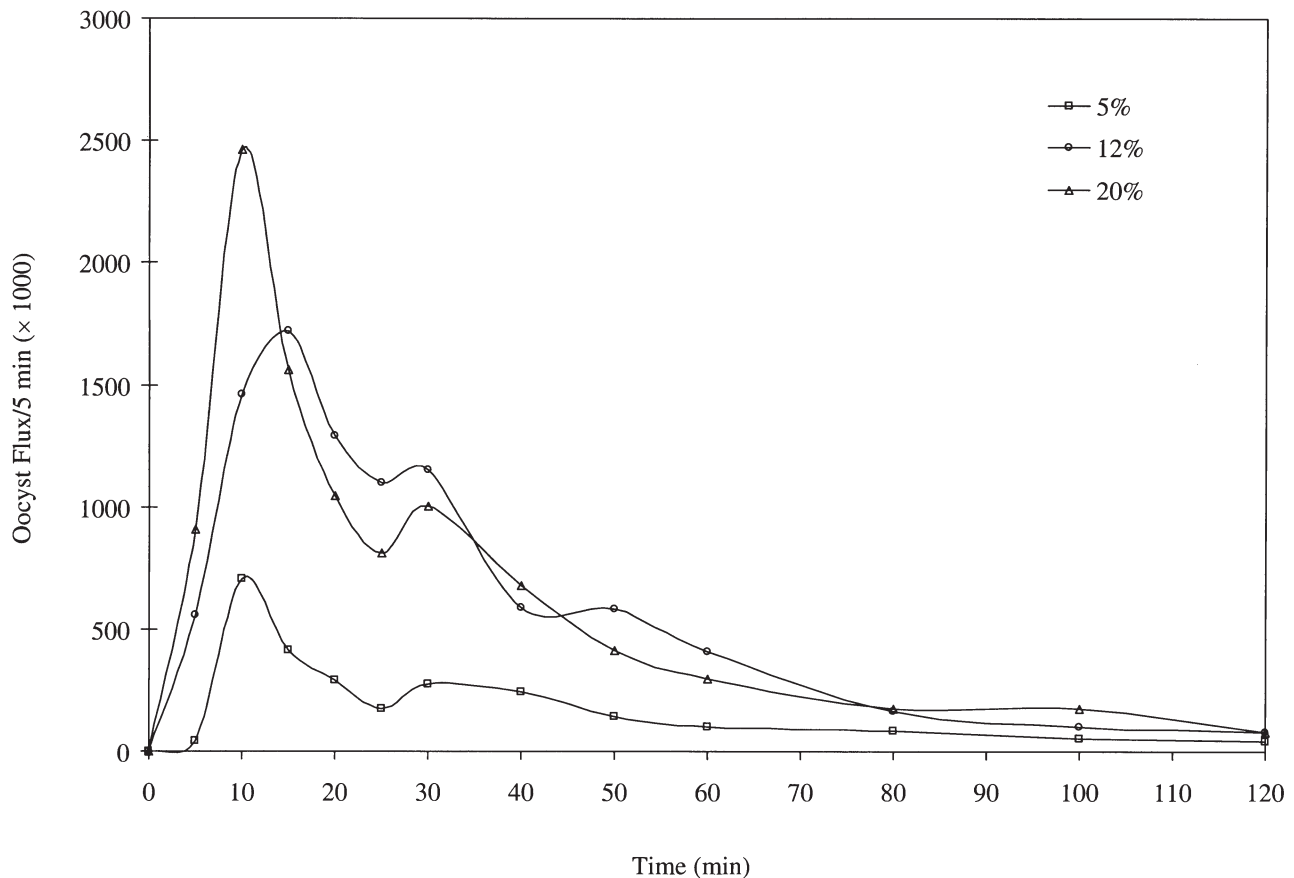


Fig. 2. Mean *Cryptosporidium parvum* oocyst flux (concentration  $\times$  flow) breakthrough curves in overland flow from 0.5-m-wide  $\times$  1.1-m-long  $\times$  0.3-m-deep soil boxes set to three land slope treatments, planted with grass, filled with a sandy loam soil, spiked with 200 g of cattle fecal deposit with  $2 \times 10^8$  oocysts, and subject to simulated rainfall intensities of 30 to 47.5 mm/h for 2 h.

The effect of land slope and rainfall application rate on the  $\log_{10}$  reduction per meter of vegetated buffer was determined using negative binomial regression (Hardin and Hilbe, 2001), with total oocyst flux set as the outcome variable, percent land slope, rainfall application rate, and rainfall emitter position (nuisance variable) functioning as covariates, and trial (1 through 4) set as a clustering variable (Stata Corporation, 2001). A forward stepping algorithm was used to build the model, with significance for inclusion in the model set at  $P$  value of  $\leq 0.10$ , based on a Wald test.

Percent recovery for the immunofluorescent microscopy procedure was estimated by fitting a negative binomial regression model (Hardin and Hilbe, 2001) to the observed number of oocysts, with the number of spiked or expected oocysts functioning as the offset (or exposure) variable. Negative binomial regression tends to provide a better fitting model compared to Poisson regression due to overdispersion of oocyst count data (Teunis et al., 1997; Atwill et al., 1998; Hoar et al., 2000).

## RESULTS AND DISCUSSION

A total of  $2 \times 10^8$  oocysts were applied to each plot. The total number of oocysts discharged from each soil box (combined overland and subsurface flow) during the 120-min simulation ranged from  $1.5 \times 10^6$  to  $23.9 \times 10^6$  oocysts. Examination of the mean breakthrough

curves of each land slope treatment demonstrated that although mean concentration of oocysts/L in overland flow was not substantially different for buffers set at different land slopes, mean rates of overland flow were positively correlated with land slope (Fig. 1). This resulted in distinct breakthrough curves for the flux (flow  $\times$  concentration) of discharged oocysts for buffers set at 5% compared to buffers set at 12 and 20% (Fig. 2). The shape of the overland flow breakthrough curves illustrates the initial detachment and flushing transport of oocysts from the fecal matrix and soil box, which is in agreement with our previous findings in the field under simulated rainfall conditions (Tate et al., 2000). In contrast, the mean breakthrough curves of each land slope for both the mean concentration of oocysts/L and subsurface flow rate were substantially different for buffers set at 5% compared to buffers at 12 and 20% land slope, resulting in distinct breakthrough curves for the subsurface flux of oocysts for buffers set at 5% compared to buffers at 12 and 20% land slope (Fig. 3 and 4). Thus, at land slopes of 12 and 20%, the majority of oocyst flux from the vegetated buffer occurred due to overland transport (Table 1). At a 5% land slope, there was substantial subsurface flux of oocysts from the vegetated buffer. It is important to note that under the

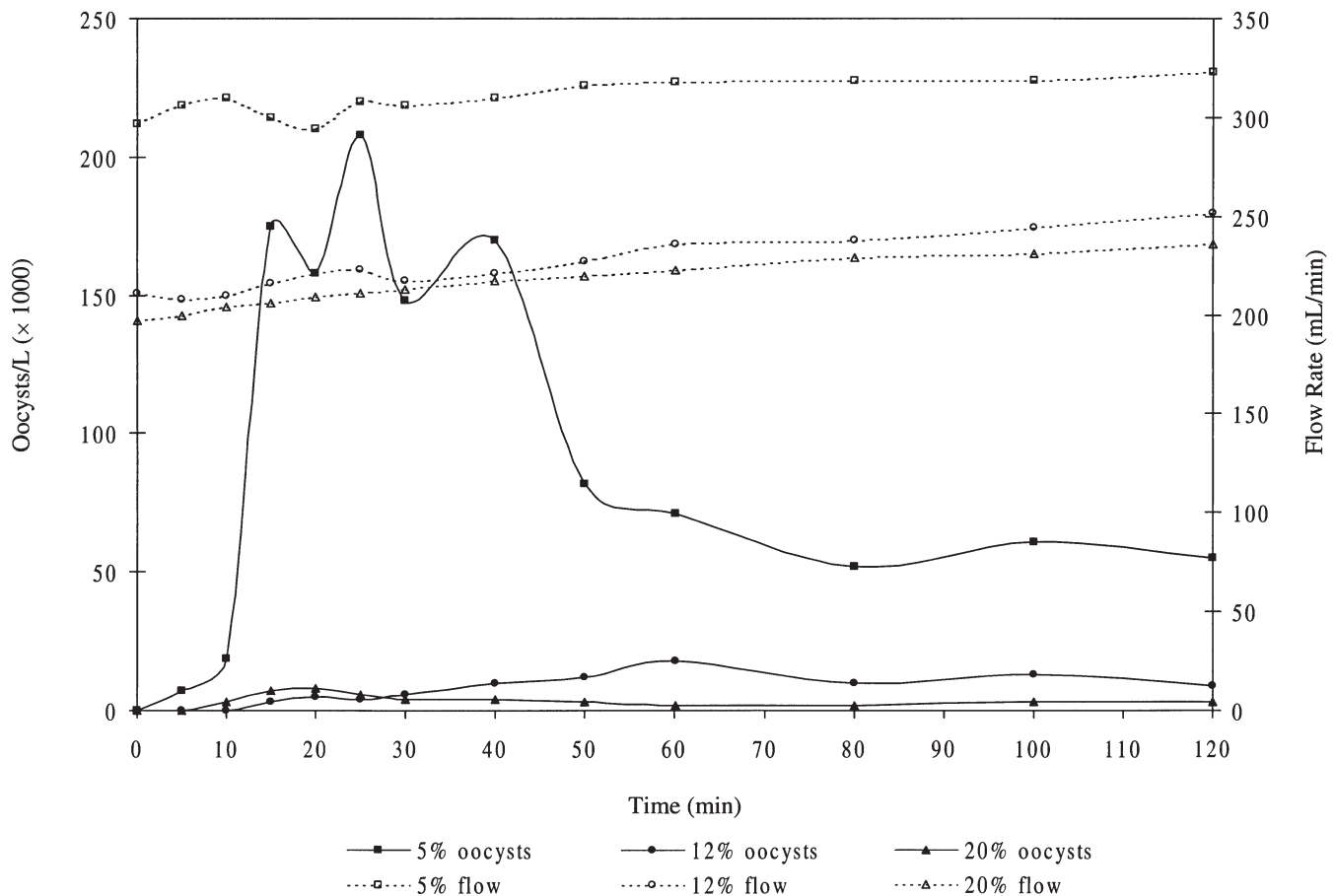


Fig. 3. Mean *Cryptosporidium parvum* oocyst concentration breakthrough curves in subsurface flow from 0.5-m-wide  $\times$  1.1-m-long  $\times$  0.3-m-deep soil boxes set to three land slope treatments, planted with grass, filled with a sandy loam soil, spiked with 200 g of cattle fecal deposit with  $2 \times 10^8$  oocysts, and subject to simulated rainfall intensities of 30 to 47.5 mm/h for 2 h.

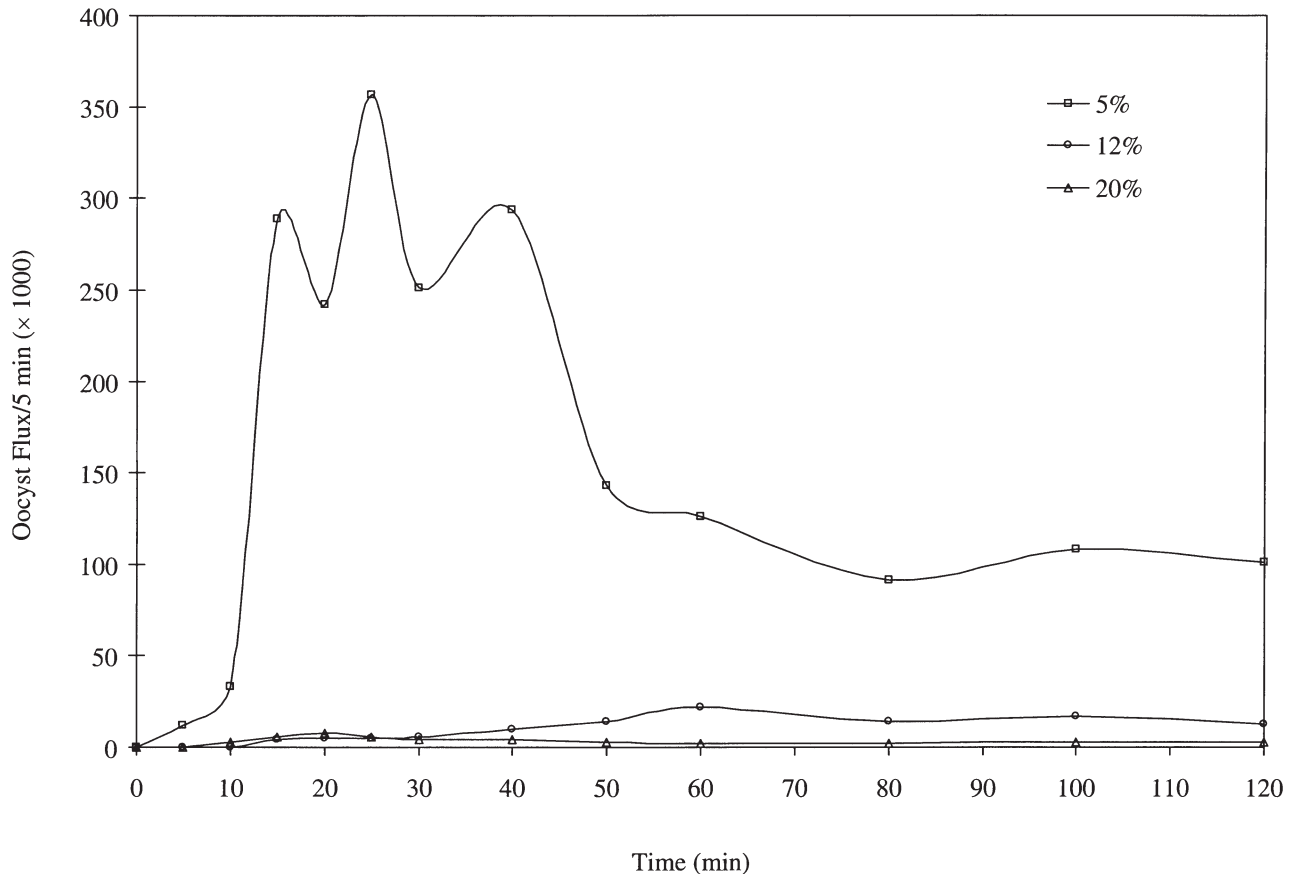


Fig. 4. Mean *Cryptosporidium parvum* oocyst flux (concentration  $\times$  flow) breakthrough curves in subsurface flow from 0.5-m-wide  $\times$  1.1-m-long  $\times$  0.3-m-deep soil boxes set to three land slope treatments, planted with grass, filled with a sandy loam soil, spiked with 200 g of cattle fecal deposit with  $2 \times 10^8$  oocysts, and subject to simulated rainfall intensities of 30 to 47.5 mm/h for 2 h.

conditions of this experiment macroporosity may have been reduced due to repacked soil conditions, leading to an overestimate of surface runoff and an underestimate of subsurface flow, thereby influencing the flow path and subsequent filtration of waterborne *C. parvum*. With this caveat in mind, the observed overall mean  $\log_{10}$  reduction of total *C. parvum* flux per meter of vegetated buffer was 1.44, 1.19, and 1.18 for buffers at 5, 12, and 20% land slope, respectively. Mean percent recovery for the direct immunofluorescent microscopy assay was 64.2% (95% CI = 56.0%, 73.6%).

A negative binomial regression model was fit to the data to statistically evaluate the effect of land slope and precipitation rate on the total flux, and thus  $\log_{10}$  reduction, of oocysts per meter of vegetated buffer estimated for each soil box (Table 2). Graphical display of the negative binomial regression model illustrates that vegetated buffers at land slopes of 12 and 20% had significantly more oocysts in their effluent compared to buffers at a 5% land slope (Fig. 5). This reduces the value for the  $\log_{10}$  reduction per meter of vegetated buffer for higher sloped buffers by about 0.4 (or 20–30%) across the range of precipitation rates when compared to the  $\log_{10}$  reduction of buffers at a 5% land slope (Fig. 5). Rainfall application rate (mm/h) was also associated with oocyst flux from these vegetated buffers,

such that for every additional mm/h applied to the soil box for a range of 30 to 47.5 mm/h and a 2-h duration, there was a concomitant decrease of 2 to 4% in the  $\log_{10}$  reduction per meter of buffer (Fig. 5). Rainfall intensities ranging from 30 to 47.5 mm/h for a 2-h duration event occur very rarely for the southern Sierra Nevada foothills, with return periods in excess of 25 yr for rainfall intensities greater than 30 mm/h.

These results suggest that each additional meter of grass vegetated buffer with  $\geq 95\%$  surface cover and under conditions of 5 to 20% land slope, sandy loam soil, and precipitation rates of 30 to 47.5 mm/h for a 2-h duration could generate an additional 0.9 to 2.0  $\log_{10}$  mean reduction in *C. parvum* oocysts relative to the total oocyst spike applied in a fecal matrix (Table 2). These values are consistent with our previous estimate of 1.0 to 1.9  $\log_{10}$  mean reduction in waterborne *C. parvum* oocysts using a meter long soil box with 85 to 99% grass cover and under conditions of 5 to 20% land slope, sandy soil, and rainfall rates of 1.5 or 4.0 mL/cm<sup>2</sup>/h (Atwill et al., 2002), and similar to the range of values observed by Trask et al. (2004) for their vegetated buffers. The substantial release of oocysts from fresh fecal deposits and the association between land slope and overland transport of oocysts during storm events is also consistent with previous findings in the field under

**Table 1. Descriptive statistics for the efficacy of a 1-m grass vegetated buffer to filter *Cryptosporidium parvum* oocysts from overland and subsurface flow from 0.5-m-wide × 1.1-m-long × 0.3-m-deep soil boxes set at three different land slopes.**

	Mean oocysts detected in discharge†	Percent of total oocyst flux	Mean log <sub>10</sub> reduction of oocysts deposited‡
	no.	%	
<b>5% Land slope</b>	<b>7.31 × 10<sup>6</sup></b>	–	<b>1.44</b>
Overland flow	3.76 × 10 <sup>6</sup>	51.4	–
Subsurface flow	3.55 × 10 <sup>6</sup>	43.6	–
<b>12% Land slope</b>	<b>12.8 × 10<sup>6</sup></b>	–	<b>1.19</b>
Overland flow	12.5 × 10 <sup>6</sup>	97.7	–
Subsurface flow	0.3 × 10 <sup>6</sup>	2.3	–
<b>20% Land slope</b>	<b>13.1 × 10<sup>6</sup></b>	–	<b>1.18</b>
Overland flow	13.0 × 10 <sup>6</sup>	99.4	–
Subsurface flow	0.1 × 10 <sup>6</sup>	0.6	–

† Four replicate soil boxes per land slope treatment.

‡ Mean log<sub>10</sub> reduction calculated for total oocysts in both overland and subsurface flow, with an initial spike of 2 × 10<sup>8</sup> *C. parvum* oocysts in a 200-g fresh fecal pat to each soil box.

natural rainfall events for annual grasslands in the southern Sierra Nevada foothills (Tate et al., 2000), as well as under laboratory conditions (Mawdsley et al., 1996a; Trask et al., 2004). Figures 1 and 3 illustrate the effect of increasing land slope on the ratio of overland to subsurface flow volumes. The significantly higher retention of oocysts at 5% land slope is likely due to a substantially higher volume of rainfall infiltrating the soil surface and becoming subsurface flow, resulting in a substantial portion of the *C. parvum* load being removed via filtration and absorption within the soil matrix (Mawdsley et al., 1996b; Harter et al., 2000).

## CONCLUSIONS

We found that a meter of grass vegetated buffer with ≥95% surface cover and under experimental conditions of 5 to 20% land slope, sandy loam soil, and 2-h precipitation rates of 30 to 47.5 mm/h produced a 0.9 to 2.0 log<sub>10</sub> mean reduction in *C. parvum* oocyst flux relative to the total oocyst load applied in a fecal matrix. The observed overall mean log<sub>10</sub> reduction of total *C. parvum* flux per meter of grass vegetated buffer was 1.44, 1.19, and 1.18 for buffers at 5, 12, and 20% land slope, respectively. These levels of log<sub>10</sub> reduction per meter of vege-

tated buffer can be matched to a mammal's specific rate of *C. parvum* loading to guide buffer width recommendations (Atwill et al., 2002). For example, we have calculated that adult beef cattle on California rangeland shed on average between 3800 and 9200 oocysts per cow per day (Atwill et al., 2003), or approximately 6.5 × 10<sup>5</sup> oocysts per day for a herd of 100 beef cattle with no calves or yearlings. Based on an estimated range of 1.2 to 1.4 log<sub>10</sub> reduction per meter of grass buffer, ≥5 m of equivalent buffer would be needed to adequately reduce the translocation potential of this herd's 24-h cumulative load of freshly deposited oocysts. Such estimates remain only crude approximations at this time, but these results in combination with previous research on vegetated buffer filtration for this protozoal pathogen (Mawdsley et al., 1996a; Tate et al., 2000; Atwill et al., 2002; Trask et al., 2004) provide insight into the inherent capacity of grasslands with sufficient infiltration rates to attenuate *C. parvum* oocysts deposited by cattle and other mammalian species. Moreover, these studies illustrate the potential for vegetated buffers to function as one of a suite of beneficial management practices that a landowner can use to minimize the risk of waterborne cryptosporidiosis in humans from livestock production systems.

**Table 2. Negative binomial regression model for the effect of percent land slope and rainfall rate on grassed vegetated buffer strip filtration of waterborne *Cryptosporidium parvum* oocysts on 0.5-m-wide × 1.1-m-long × 0.3-m-deep soil boxes.**

Factor	Regression coefficient for mean number of nonfiltered oocysts†	90% CI for regression coefficient	P value‡
<b>Land slope</b>			<b>0.001</b>
5%§	–	–	–
12%	0.83	(0.03, 1.63)	0.086
20%	1.02	(0.65, 1.38)	0.001
<b>Rainfall rate (mm/h)</b>	<b>0.91</b>	<b>(0.40, 1.42)</b>	<b>0.004</b>
<b>Position soil box¶</b>			<b>0.013</b>
A§	–	–	–
B	0.17	(–0.58, 0.92)	0.72
C	–0.67	(–1.04, –0.29)	0.003
<b>Intercept</b>	<b>11.79</b>	<b>(10.1, 13.5)</b>	<b>0.001</b>

† Total nonfiltered oocysts set as the outcome variable, percent land slope, mean precipitation rate, and rainfall emitter position (nuisance variable) set as covariates, and trial (1 through 4) set as a clustering variable to adjust standard errors for potential intra-trial dependencies. Precipitation rate measured as the total volume of water in both overland and subsurface flow for the 120-min experiment divided by 2 h × 5000 cm<sup>2</sup> box surface area, then adjusting for mm/h.

‡ Significance (regression coefficient ≠ 0) was determined at a P value of ≤0.10.

§ Referent condition to which other levels of the categorical factor are compared.

¶ Each soil box assigned randomly to one of three positions under the rainfall emitter, with slight differences in precipitation rates between emitters (i.e., a nuisance variable).

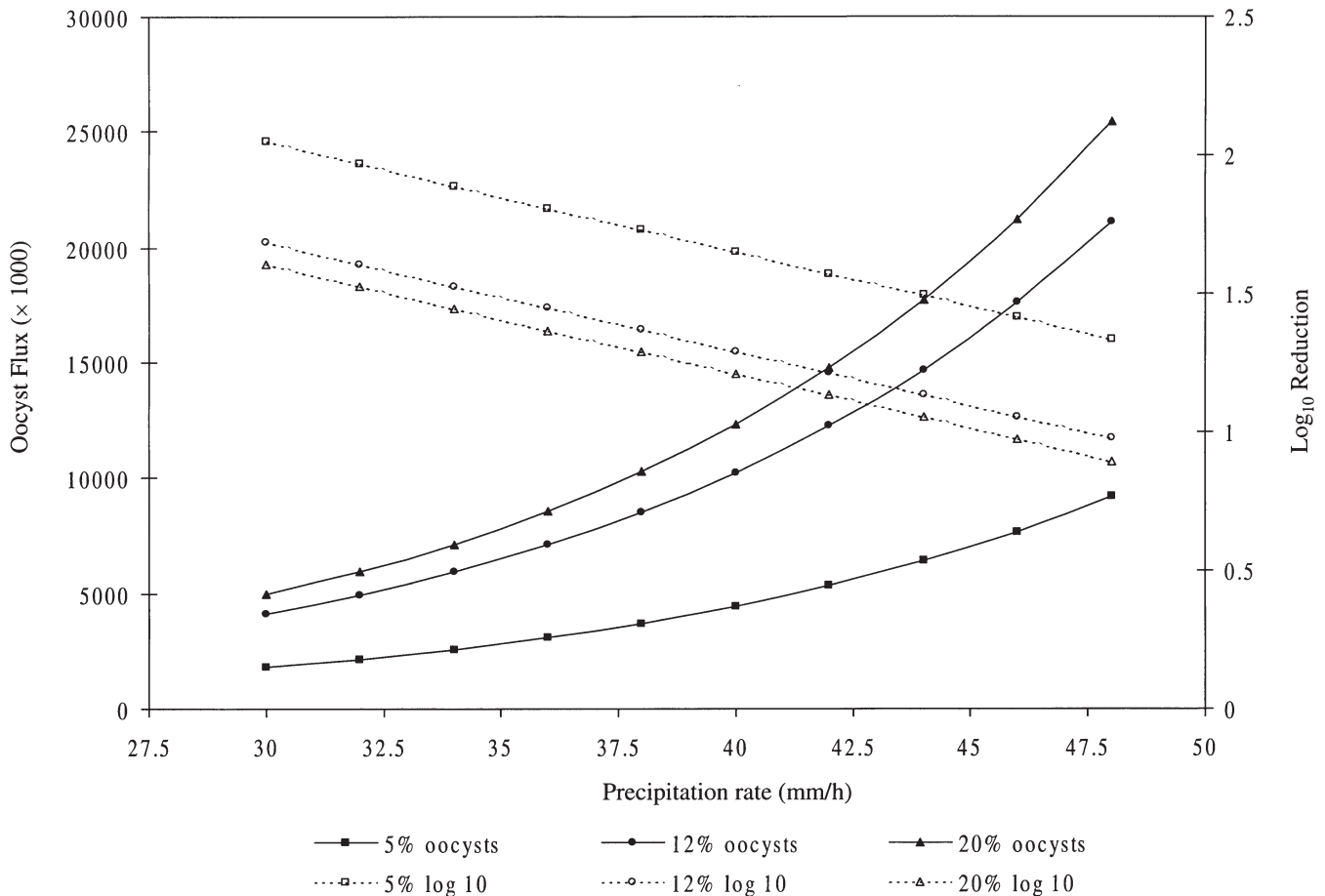


Fig. 5. Graphical display of a negative binomial regression model quantifying the effect of land slope and precipitation rate on the total *Cryptosporidium parvum* oocyst flux, and thus  $\log_{10}$  reduction, of oocysts per meter of vegetated buffer with  $\geq 95\%$  grass cover, sandy loam soil, and *C. parvum* application in a cattle 200-g fecal deposit spiked with  $2 \times 10^8$  oocysts.

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