Role of irrigation and wastewater reuse: comparison of subsurface irrigation and furrow irrigation

C. Choi*, I. Song*, S. Stine**, J. Pimentel* and C. Gerba**
* Dept. of Agri. and Biosystems Engineering, Univ. of Arizona, Tucson, AZ 85721, USA
  (E-mail: cchoi@arizona.edu)
** Dept. of Soil and Water Environ. Science, Univ. of Arizona, Tucson, AZ 85721, USA
  (E-mail: gerba@ag.arizona.edu)

Abstract Two different irrigation systems, subsurface drip irrigation and furrow irrigation, are tested to investigate the level of viral contamination and survival when tertiary effluent is used in arid and semi-arid regions. The effluent was injected with bacteriophages of PRD1 and MS2. A greater number of PRD1 and MS2 were recovered from the lettuce in the subsurface drip-irrigated plots as compared to those in the furrow-irrigated plots. Shallow drip tape installation and preferential water paths through cracks on the soil surface appeared to be the main causes of high viral contamination in subsurface drip irrigation plots, which led to the direct contact of the lettuce stems with the irrigation water which penetrated the soil surface. The water use efficiency of the subsurface drip irrigation system was higher than that of the furrow irrigation system. Thus, subsurface drip irrigation is an efficient irrigation method for vegetable crops in arid and semi-arid regions if viral contamination can be reduced. Deeper installation of drip tapes, frequent irrigations, and timely harvests based on cumulative heat units may further reduce health risks by ensuring viral die-off under various field conditions.

Keywords Irrigation; lettuce; microbial contamination

Introduction

Growing competition for scarce water resources coupled with laws limiting groundwater use have led to efforts for agricultural wastewater reuse in arid and semi-arid lands. However, reclaimed water reuse for irrigation water may escalate the risks of food contamination, resulting in restriction of its use for agricultural purposes by guidelines which might be unnecessarily conservative (Shuval, 1991; WHO, 2000). Therefore, properly developed guidelines based on scientific research regarding safety issues should be developed to achieve safe resource management.

It has been reported that many food-borne outbreaks were associated with enteric viruses which have the ability to survive in harsher environments compared to bacteria (Seymour and Appleton, 2001). Important factors affecting viral survival include temperature, soil moisture content, adsorption to soil particles, pH, solar radiation, and soil type (Yates et al., 1988; Seymour and Appleton, 2001). Hurst et al. (1980) reported that temperature and soil moisture levels appeared to be the most important factors affecting viral inactivation in soil. However, viral survival cannot be addressed explicitly in closed form since much of the known information is qualitative in nature and not much of the data are available (Yates et al., 1988). Moreover, temperature and soil moisture content in field conditions change continuously. Microbial survival models developed from constant conditions have limitations in their application to the prediction of viral survival under natural conditions (Hurst et al., 1980).

The concepts of “heat units” or “degree days” have been used for predicting harvest time for various crops and pest management (Perry et al., 1997). Arnold (1960) showed that the sine curve method resulted in similar values of degree days to those from diurnal
temperature curve. The daily maximum and minimum temperature method was suggested as a simple method for calculating cumulative degree days by Baskerville et al. (1968) and Snyder et al. (1985).

It has been suggested that subsurface drip irrigation systems have advantages over other methods in terms of reducing health risks from reclaimed water reuse by minimizing the exposure of the irrigated water to people or agricultural produce as well as filtering potential pathogens by soil (Enriquez et al., 2003; Oron et al., 1992, 1995; Alum et al., 2000). In addition to examining the contamination level of subsurface drip irrigation, the survival of viruses in the field must be investigated.

The primary objective of this study was to compare subsurface drip irrigation and furrow irrigation systems in terms of viral contamination. The efficacy of the application of the heat unit concept to predicting viral survival was also investigated.

**Methods**

The field experiment was conducted at the Campus Agricultural Center of the University of Arizona. Eight experimental plots were prepared with four plots of each irrigation system, subsurface irrigation (SDI) and furrow irrigation (FI). The experimental plots were plowed to depths of 30–40 cm and the beds were listed and shaped in a north-south orientation. Drip tapes were installed in the middle of the beds at a depth of 20 cm in SDI plots. Iceberg lettuce was seeded, two rows per bed with 30 cm spacing, and irrigated with chlorinated tertiary-treated domestic wastewater.

MS2 stock serially diluted to an approximate concentration of $10^5$ pfu/ml was mixed with 0.2 ml of host, *Escherichia coli* (ATCC 15597) in growth phase, in molten top agar and plated onto presolidified TSA (tryptic soy agar) Petri dishes. After 18 to 24 hr incubation, 6 ml of Tris buffer was added to the Petri dishes and the dishes sat for three hours to allow the phage to diffuse into the Tris buffer. The liquid fraction was collected from dishes and centrifuged at 4,000 rpm. The resulting supernatant was filtered using a sterilized 0.2 µm filter to remove the host and stored at 4°C until used. PRD1 was prepared in the same manner as MS2 with the exception of using *Salmonella typhimurium* LT2 as the host.

Before injecting the bacteriophage, background samples were taken. The tertiary effluent from Roger Road Wastewater Treatment Plant in Tucson was irrigated after dechlorination using sodium thiosulfate. A DPD colorimetric test (Hach Chemical Co.) was conducted to make sure that no free chlorine was present in irrigation water. For each irrigation system, a 20 L reservoir was filled with irrigation water and seeded with the prepared PRD1 or MS2 and stirred for 10 minutes before injection. The reservoir contents were injected into the irrigation line using a pump and distributed equally between each plot.

Lettuce samples were collected randomly from each of the eight plots. The lettuce was cut from the stem and the 2–3 outermost leaves discarded. Soil samples were collected in a randomly chosen location within the plot: in-between the beds for furrow plots, and on the beds for subsurface drip plots. A small chisel was used to collect the surface soil samples and a bucket auger was used to collect the soil samples at a depth of 10 cm. All equipment used for sampling was disinfected with 30% bleach in between each sample collection. The samples were placed and stored in an ice chest until assayed in the laboratory.

The dimensions and weight of lettuce were measured before processing. The leaves removed from the outer layer were torn into smaller pieces and placed inside a plastic bag with 100 ml of 3% beef extract. Samples were shaken for 20 minutes in an incubator after which the solution was transferred to a 50 ml centrifuge tube. Approximately 10 g of each soil sample was weighed and placed overnight in an oven at 104°C to measure soil moisture content. 5 g soil from each sample was placed in a 250 ml bottle with 45 ml of 3% beef extract.
extract. After stirring for 30 minutes the samples were centrifuged at 4,000 rpm. The supernatant was transferred to a 50 ml centrifuge tube after 30 minutes of stirring, followed by centrifuge. Solutions from lettuce and soil samples were adjusted to a pH of 7–8.

MS2 was assayed using the plaque forming method with the bacterial host *Escherichia coli* ATCC15597 (Governal and Gerba, 1997). The same method was used for PRD1 assay, with the exception of using the bacterial host *Salmonella thyphimurium* LT 2.

**Results and discussion**

The experimental data of the past two years (2001 and 2002) are summarized in Figure 1. Greater contamination of lettuce took place on SDI plots compared to FI plots. This can be explained by contact mechanisms of irrigated water with lettuce in SDI plots. It was observed in both experiments that water irrigated through subsurface drip lines came directly out of the seedbeds so as to come in contact with the lettuce stems. Loose soil due to plowing in the first year experiment might decrease the soil filtration of viruses and make it easier for irrigation water to flow preferentially though the soil to the surface. In spite of the natural soil compaction for a year, the ineffective displacement of the preferential water paths might be the cause of the greater contamination of lettuce on SDI plots. Therefore, two possible practices were suggested in order to reduce direct contact of irrigation water with plants and minimize soil surface wetting: bury drip tapes deep enough to minimize surface wetting from irrigation water, or irrigate more frequently if the depths of crop root are shallow.

MS2 and PRD1 generally experienced quicker die-off in the first year experiment than in the second year experiment, possibly due to higher temperatures during the first year. The second year experiment was delayed because of hail storms and was completed later in the year. Accordingly, temperature readings were lower than those of the first year. The viral number of subsurface soil samples increased slightly after rainfall. This might result from the transport of the viruses on the surface into the subsurface along with rainfall infiltration.

The inactivation rates were expressed in the following form:

\[
\frac{N_t}{N_0} = 10^{-kd t}
\]

where \( t, N_r, N_0, \) and \( kd \) represent time (days), microbial number at \( t \) days, initial microbial number, and inactivation rate \( (1/\text{days}) \), respectively. The corresponding \( kd \) values are summarized in Table 1. Inactivation rates from lettuce samples from FI plots in 2001 and soil samples at 10 cm from FI in 2002 could not be estimated. The numbers of viruses present from those samples were too low to observe any trend of inactivation. General trends of inactivation were observed, although some of the regression results have low determination values \( (R^2) \).

Overall, greater inactivation rates were observed on the lettuce than in the soil as well as on the soil surface compared to the subsurface, which is as expected. Viruses in the soil have more protection from adverse environmental conditions such as solar radiation and desiccation than those on lettuce. In general, the data from furrow surface showed higher regression coefficients. The conditions of the furrow surface may be relatively uniform in terms of viral distribution as well as the level of exposure to other environmental factors during irrigation as compared to SDI and subsurface conditions.

ANOVA tests were conducted using surface soil sample data, which were relatively higher in \( R^2 \) values. The results showed that the difference between the inactivation rates of 2001 and 2002 data were statistically significant for both of MS2 and PRD1 (P value < 0.05) whereas inactivation rates between MS2 and PRD1 were not significantly different.
in either experiments ($p > 0.05$). The former probably resulted from temperature differences, as temperatures for the second experiment in 2002 were lower than in 2001. Inactivation rates between SDI and FI were not significantly different.

Although temperature has been known as one of the most significant factors influencing microbial survival, continuous change of temperature in field conditions has made it difficult to consider temperature itself as a single factor in the prediction of microbial survival. In the context of this problem, degree days, which is the sum of the difference between lower threshold temperature and maximum temperature, can be a reasonable variable incorporating daily temperature changes into a factor.

The AZMET weather data for the period of the experiment is summarized in Table 2. The experiments in 2001 were carried out in warmer temperatures and relatively dry conditions, especially the PRD1 experiment. In contrast, 2002 experiments were conducted in colder and more humid condition than in 2001. The daily temperature data of AZMET were used for estimating degree days. Of several methods of calculating degree days, the new

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>MS2</th>
<th>PRD1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
<td>2002</td>
</tr>
<tr>
<td></td>
<td>$K_d$ (day$^{-1}$)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>LD</td>
<td>0.5455</td>
<td>0.8825</td>
</tr>
<tr>
<td>LF</td>
<td>NA</td>
<td>0.5763</td>
</tr>
<tr>
<td>DS</td>
<td>0.2308</td>
<td>0.8363</td>
</tr>
<tr>
<td>DB</td>
<td>0.1527</td>
<td>0.7024</td>
</tr>
<tr>
<td>FS</td>
<td>0.2895</td>
<td>0.9537</td>
</tr>
<tr>
<td>FB</td>
<td>0.1519</td>
<td>0.5130</td>
</tr>
</tbody>
</table>
method (Snyder, 1985) based on the trigonometric sine curve method introduced by Arnold (1960) and later refined by Baskerville and Emin (1965) was utilized. This method approximates diurnal ambient temperatures by using daily maximum (T\text{MAX}) and minimum (T\text{MIN}) temperatures and a sine curve as following:

\[ T = M + W \sin(t) \]  

(2)

where \( T \) = diurnal temperatures (°C), \( M = (T\text{MAX} + T\text{MIN})/2 \), \( W = (T\text{MAX} + T\text{MIN})/2 \), and \( t = \pi (\text{hours} - 6)/12 \) or time in radians from \(-\pi/2\) to \(3\pi/2\). Degree days can be calculated by integrating Eq. (2) and applying threshold temperatures.

\[ DD = \left[ (M - THR)(\pi/2 - \theta) + W \cos(\theta) \right]/\pi \]  

(3)

where \( THR \) = lower threshold temperature, \( \theta = \arcsin[(THR - M)/W] \) = the time when the sine curve intersects THR.

Here, 4°C was chosen as a lower threshold temperature without an upper threshold temperature. Below this temperature, minimal microbial inactivation takes place (Seymour and Appleton, 2001), and inactivation will increase with the increase of temperature. To predict a certain degree of viral inactivation in terms of degree days, it was necessary to derive a parameter such that its unit includes a time component. Therefore, the cumulative degree days were first estimated for each experiment and then averaged throughout the period (Table 3). Average degree days reflect temperature changes consistent with AZMET weather data whose temperatures for the PRD1 experiment in 2001 were relatively higher than those for the other experiments. Therefore, average degree days incorporated with temperature changes can be a reasonable parameter in predicting microbial survival.

Cumulative degree days required for 99.9% viral die-off were calculated by multiplying the average degree days by the time to reach 3 log reduction of the initial viral numbers using Eq. (1). Figure 2 shows that the cumulative degree days for the 3 log reduction of viruses vary with experimental conditions. This indicates that viral survival cannot be explained by a single factor, such as temperature. Nonetheless, temperature is the most important environmental parameter for viral inactivation. The significance of the presented data is that cumulative degree days are associated with the persistency of viruses under the given condition. In other words, the higher the persistency of a virus, the greater the heat

<table>
<thead>
<tr>
<th>Year</th>
<th>Virus</th>
<th>Periods (days)</th>
<th>Rainfall (mm)</th>
<th>Daily air temp (°C)</th>
<th>RH (%)</th>
<th>Soil* temp. (°C)</th>
<th>Solar radiation (MJ/m²)</th>
<th>Degree days**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>PRD1</td>
<td>19</td>
<td>1.016</td>
<td>29.4</td>
<td>0.6</td>
<td>16.6</td>
<td>15.6</td>
<td>249.7</td>
</tr>
<tr>
<td></td>
<td>MS2</td>
<td>11</td>
<td>6.096</td>
<td>25.0</td>
<td>-3.3</td>
<td>8.5</td>
<td>9.3</td>
<td>63.1</td>
</tr>
<tr>
<td>2002</td>
<td>PRD1</td>
<td>18</td>
<td>14.224</td>
<td>23.3</td>
<td>-3.9</td>
<td>7.4</td>
<td>7</td>
<td>93.4</td>
</tr>
<tr>
<td></td>
<td>and MS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Soil temperature at 4 inches deep and ** Threshold temperature = 4°C

<table>
<thead>
<tr>
<th>Year</th>
<th>Virus</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS2</td>
<td>PRD1</td>
</tr>
<tr>
<td></td>
<td>Cumulative degree days (°C)</td>
<td>63.1</td>
<td>249.7</td>
</tr>
<tr>
<td></td>
<td>Experiment period (days)</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Average degree days (°C/days)</td>
<td>6.3</td>
<td>13.9</td>
</tr>
</tbody>
</table>
unit required to inactivate to the 99.9% level. Along with this concept, both of the viruses appeared less persistent on lettuce than on or in soils, which is consistent with experiment results showing a faster die-off rate on lettuce. Viruses on surface soil, regardless of irrigation systems, have similar heat unit requirements. The exception is PRD1 data in 2001. Although furrow subsurface data in 2002 were not available due to low contamination, viruses appeared to be more persistent in subsurface soil, as anticipated, without a significant difference between the two irrigation systems. MS2 in soil showed longer longevity in 2002 than in 2001 while the opposite was true for PRD1. Considering the parameter of relative humidity between the two experiments, the results indicate that MS2 is more persistent in humid conditions than in dry conditions, in contrast to PRD1 which is more persistent in dry conditions. PRD1 is more tolerant than MS2 to higher temperatures.

Figure 3 compares the average cumulative degree days required for 3 log reduction between MS2 and PRD1. PRD1 requires greater cumulative heat unit than compared to MS2. Again, the only environmental factor affecting viral survival taken into account was temperature.

Conclusions
A greater number of PRD1 and MS2 were recovered on the lettuce from SDI plots than from FI plots due to direct contact of the irrigated water with lettuce stems. It is possible that loosened soil from first-year plowing weakened the filtration function of the soil and resulted in higher contamination. During the second year the preferential water paths from the previous experiment were not effectively displaced and the irrigated water was observed coming out of the beds in the SDI plots. Deeper installation of drip tapes and more frequent irrigations were suggested in order to minimize soil surface wetting in SDI plots.
and reduce potential contamination. In particular, frequent irrigation at the end of the growing season may drastically reduce food contamination. Deeper lettuce roots at the end of the growing season may accommodate lower wet zones without sacrificing water use efficiency. Such practices may guarantee dry surfaces and can be particularly useful to prevent health risks when wastewater is used for irrigation in arid and semi-arid regions.

The inactivation rates of bacteriophages were evaluated and used in calculating the time required for 99.9% die-off of viruses as well as the cumulative degree days in combination with average degree days. Greater cumulative heat units in the subsurface were required for 99.9% viral reduction and a lesser amount on the lettuce, which makes sense since the subsurface condition may supply protection for viruses from harsher conditions. MS2 is more persistent in humid conditions than in dry conditions, in contrast to PRD1 which shows more persistence in dry conditions. PRD1 is more tolerant to higher temperatures than MS2 and showed higher heat requirements for 99.9% die-off. Average degree days can be a reasonable parameter in predicting virus survivability.

The present research focused on the estimation of virus die-off time, introducing the heat unit concept, because temperature is directly related to viral survivability. Other significant factors such as soil moisture content, solar radiation, and relative humidity need to be incorporated in the model to predict viral survivability.

Acknowledgement
This research and development was supported in part by funds provided to the International Arid Lands Consortium (IALC) by the USDA Forest Service and by the USDA Cooperative State Research, Education, and Extension Service.

References