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Comparison of sulfur hexafluoride, fluorescein and rhodamine dyes and the bacteriophage PRD-1 in tracing subsurface flow

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Abstract

We compared velocities of the subsurface flow from a mounded onsite septic system towards a depressional wetland with three types of tracer; an inert gas, sulfur hexafluoride (SF_6), two fluorescent dyes, fluorescein and rhodamine WT, and a viral tracer, the bacteriophage PRD-1. The movement of both fluorescent dyes was significantly retarded in the soils compared to both SF_6 and PRD-1. In experiments using injection solutions containing both a dye and SF_6, fluorescein was found to move at least 3–4 times slower than SF_6, and rhodamine was not observed away from the drainfield. In contrast, the velocities calculated from SF_6 data are very similar to the velocities calculated from the PRD-1 data obtained during the same experiment. At a second site, the movement of fluorescein was half as fast and not as extensive as the movement of SF_6. The results of these experiments indicate that fluorescent dyes may underestimate velocities of effluent from septic systems adjacent to seasonal wetlands. In contrast, SF_6 was found to perform similarly to the viral tracer PRD-1.
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1. Introduction

Onsite sewage treatment and disposal systems, a common means of wastewater treatment in Florida, are the most frequently reported source of groundwater contamination (Yates, 1985). In 1990, 30% of Florida's population used septic systems, discharging an estimated 450 million gallons of wastewater per day (FDOH, 1999). Current Florida Department of Health regulations require a 2 ft separation from the high water table to the bottom of the drainfield to allow for proper treatment of the effluent. Since the high water table is often close to the soil surface in Florida, drainfield mounds are a common solution to obtain this separation. However, the use of drainfield mounds allows for the installation of onsite sewage systems in areas previously considered too wet for traditional non-mounded systems. Often mounded systems are installed near depressional wetlands,

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known as seasonally inundated areas (SIAs). As part of a study initiated by the Florida Department of Health, the flow of onsite sewage system effluent from a mounded drainfield towards an SIA was studied using three types of tracers: an inert gas, sulfur hexafluoride (SF_6), two fluorescent dyes, fluorescein and rhodamine WT, and a viral tracer, the bacteriophage PRD-1. The objective of this paper is to compare the velocities obtained by tracing effluent from mounded septic systems using SF_6, PRD-1 and fluorescein and rhodamine dyes.

One of the most unequivocal ways to ascertain the hydraulic properties of an aquifer, velocities and pathways through a hydrological system, or to link specific sites of contamination to discharge points is via artificial tracers. In this study, the conservative groundwater tracer SF_6 was employed as the primary tracer to evaluate subsurface flow direction and velocity in two experiments at the primary site and one experiment at another site. Sulfur hexafluoride is very unreactive and detectable at low concentrations, but has the potential for degassing in mounded onsite sewage systems and low water tables (Corbett et al., 2000). Due to this potential, fluorescent dyes were used as relatively inexpensive backup tracers. Fluorescent dyes need to be used with caution as they are known to adsorb to subsurface media (Kasnavia et al., 1998; Trudgill, 1987; Omoti and Wild, 1979; Smart and Laidlaw, 1977). Fluorescein was used at both sites and rhodamine during the second experiment at the primary site. Rhodamine was used as the secondary tracer due to the presence of fluorescein in the well field at the start of the second experiment. The use of fluorescent dyes as secondary tracers allows for direct comparison of these results to previous studies. County and state health departments and others have often used fluorescent dyes to study septic systems and investigate complaints due to the relative low cost and availability compared to other tracers.

In addition to SF_6 and rhodamine WT, the tracer solution for the second experiment at the primary site contained the biological tracer PRD-1. The addition of PRD-1 was used to model migration velocities of human viruses and also allowed for a direct comparison of PRD-1 and SF_6. In several previous studies conducted in the Florida Keys, groundwater velocities were at least an order of magnitude higher for PRD-1 than those found in SF_6 experiments (Table 1). While none of these studies were conducted at the same time and in exactly the same place, they were all conducted in the limestone terrain of the Florida Keys and should be roughly comparable. However, spatial and temporal variation may account for the differing velocities. This study offers a direct comparison of PRD-1 and SF_6 by employing the two tracers concurrently.

Table 1
Groundwater velocities in the karst subsurface of the Florida Keys

Reference	Location	Tracer	Velocity (m/d)
Dillon et al. (1999)	Key Largo	SF_6	5–79
	Big Pine Key	SF_6	3–32
Dillon et al. (2000)	Long Key	SF_6	1–42
Dillon et al. (2003)	Key Colony Beach	SF_6	0.2–190
Paul et al. (1995)	Key Largo	PRD-1	14–580
Paul et al. (1997)	Key Largo	PRD-1	60–840
Paul et al. (2000)	Boot Key Harbor	PRD-1	41–1380
	Saddlebunch Keys	PRD-1	1603–3384

Characteristics of these tracers are as follows.

1.1. Sulfur hexafluoride

Sulfur hexafluoride is a water-soluble gas that is biologically and chemically inert, has a low background atmospheric concentration (10^{-15} mol/l), and can be detected at extremely low concentrations (10^{-16} mol; Wanninkhof et al., 1985). The strong potential of SF_6 as a geothermal and groundwater tracer has also been reported (Upstill-Goddard and Wilking, 1995; Wilson and Mackay, 1993) and has been used successfully in karst limestone (Dillon et al., 1999) and shallow, sandy aquifers (Corbett et al., 2000).

1.2. Fluorescein

Sodium fluorescein ($C_{20}H_{10}O_5Na_2$), a highly water soluble fluorescent dye, is bright yellow-green to the eye and has a maximum excitation of 491 nm and maximum emission of 513 nm. Many groundwater-tracing studies have employed this dye since it is inexpensive, easily detectable, non-toxic, and stable over time (Gaspar, 1987; Smart and Laidlaw, 1977). However, the dye will break down if exposed to direct sunlight.

1.3. Rhodamine WT

Rhodamine WT ($C_{29}H_{29}O_5N_2Na_2Cl$) is another fluorescent dye commonly used in groundwater systems. Rhodamine WT is bright orange with an excitation wavelength of 555 nm and an emission wavelength of 580 nm, and is inexpensive, easily detectable, non-toxic and stable over time (Sabatini and Austin, 1991; Smart and Laidlaw, 1977). The fluorescent signature of rhodamine WT is distinct from fluorescein, enabling detection of both dyes in the same system.

1.4. Bacteriophage PRD-1

The bacteriophage PRD-1 is a virus that infects the bacterium *Salmonella typhimurium* as its host. PRD-1 has been used as a viral ground water tracer in a number of studies and serves as a surrogate for human pathogenic viruses (Blanc and Nasser, 1996; Bales et al., 1995; Ryan et al. 1999). PRD-1 has also been successfully used as a groundwater tracer in the Florida Keys (Paul et al., 1995, 1997). It is an icosahedral lipid containing bacteriophage with a diameter of 62 nm (Olsen et al., 1974). Several aspects of this organism make it useful as a virus transport model: its size and transport properties are similar compared to human enteric viruses, detection methodology is relatively inexpensive and easy to perform, it is not commonly found as a natural inhabitant of environmental waters, it is harmless to humans, animals or plants, and it is rather persistent once introduced to groundwater aquifers.

2. Methods

2.1. Site description

Two tracer experiments were conducted at the primary site located at a single-family residence in Maxville, Duval county Florida. The onsite sewage system has a mounded drainfield approximately 5 m from the SIA. The mound system was located adjacent to a transitional area between two different soil types. The soil under the mound was a Mascotte-like soil, which exhibits a spodic layer. Mascotte soil is described in the NRCS Duval County Soil Survey as #38 Mascotte fine sand, 0–2 percent slopes, and described in the detailed Soil Map Units as having a natural drainage of moderately slowly permeable to moderately permeable.

The soils between the mounded drainfield and the SIA consist of approximately 1–1.5 m of fine to medium sand over a clay-rich layer. Above this clay lens, groundwater forms a saturated zone, which typically flows from the drainfield towards the SIA. The flow direction was determined by measuring the depth to the water table at several points in the study area to determine the hydraulic head prior to well installation. Samples were taken from this shallow saturated zone. The soils in the SIA are mapped in the NRCS Duval County Soil Survey as #66 Surrency loamy fine sand, depressional, 0–2 percent slopes, and described in the detailed Soil Map Units as having a natural drainage setting of 'very poorly drained'. Surrency soils are also listed as being hydric. The percent loss on ignition (LOI) of the top meter of the soil was 3.6 ± 2.5% $n = 3$.

A secondary site was located at a single-family residence in the subdivision of Brook Point in Alachua County. The soils between the drainfield and SIA were similar to the primary site with 0.5–2 m of fine to medium sand, forming a saturated zone over a clay-rich layer. The drainfield is located approximately 13.5 m from an SIA mapped in the NRCS Alachua County Soil Survey as #22 Floridana sand, depressional, and described in the detailed Soil Map Units as a 'very poorly drained soil in seasonally ponded, depressional areas and swamps.' The LOI of the top meter of the soil was 1.6 ± 1.3% $n = 3$. The height of the mound, 2 m, is approximately twice the height of the mound at the Maxville site.

At each study site, two types of monitoring wells were installed: slotted wells and multi-level sampling (MLS) wells. A licensed surveyor located the drainfield, sampling wells, the SIA edge, and recorded the elevations. Department of Health staff surveyed the well elevations at the Maxville site. The wells at the Maxville site are notated by 'Ds', for Duval County-south, and the Brook Point wells are notated with 'A', for Alachua county. After the site location, the MLS wells are indicated by 'M' and the slotted wells by 'S' followed by well number. For example, DsM2 is the MLS well 2 at the Maxville site and AS1 is slotted well 1 at the Brooke Point site.

During this study, Florida was under drought conditions and the groundwater table was depressed. Often only one or two ports of the MLS wells would yield enough water to sample. For simplicity the vertical component of the experiments is omitted. The MLS wells proved advantageous during these dry conditions, as less groundwater was required to obtain samples than with the slotted wells.

2.2. Slotted sampling well installation

Both sites were instrumented with 10 slotted wells to characterize ground water quality and measure water levels between the drainfield and the adjacent SIA. The well pattern included wells located near the toe of the drainfield mound and wells that fanned out in a wider array approaching the SIA (Fig. 1). Wells were installed to the top of the clay layer using a hand auger, typically to a depth of 1–2 m. The bottom 0.5 m of the well consisted of PVC well screen with a solid casing attached extending to the ground surface. After the well was placed at the appropriate depth, a sand pack with a bentonite plug was placed in the upper portion of the well bore. Water quality data from the slotted wells were considered in installing the more numerous MLS wells.

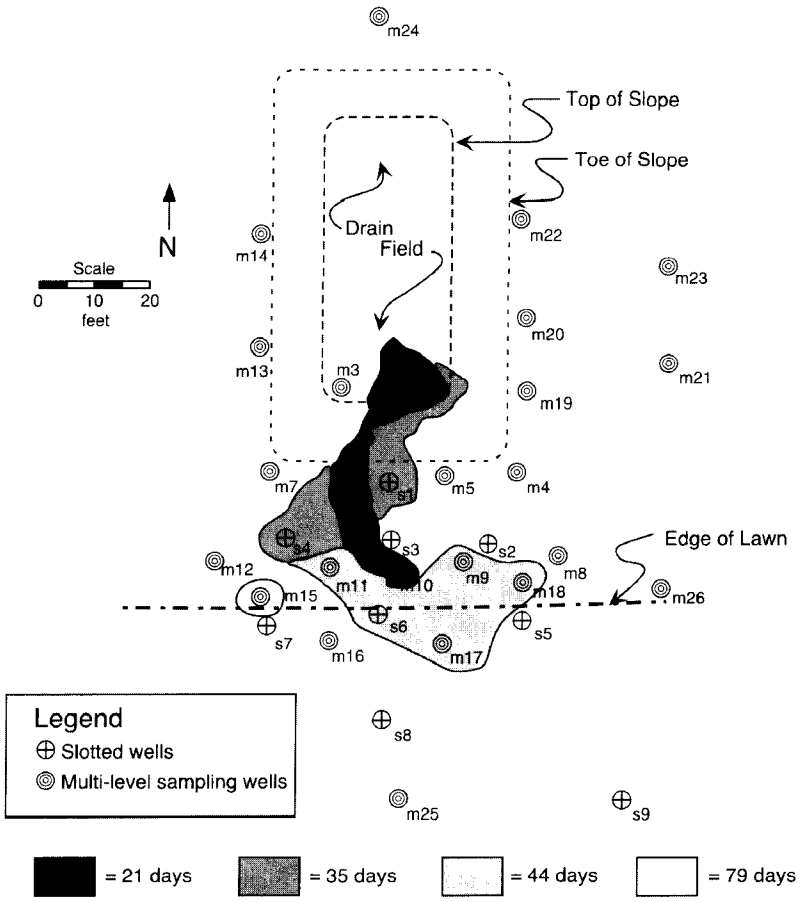

Fig. 1. Schematic diagram of the Maxville site in Duval County, Florida. The SIA originally extended into the present lawn area. The shaded areas indicate the number of days until first detection of the PRD-1 tracer in respective wells. Contours are approximate based on PRD-1 positive wells. The wells at this site are notated by Ds, for Duval County-south. After the site location, the MLS wells are indicated by M and the slotted wells by S followed by well number.

Table 2
Summary of tracer experiments conducted at the two study sites

Site	Date of injection	Tracer
Maxville, Duval County	December 3, 1999	SF_6, fluorescein
	September 19, 2000	SF_6, PRD-1, rhodamine
Brook Point, Alachua County	December 20, 1999	SF_6, fluorescein

2.3. Multi-level sampler well installation

Slotted wells tend to provide integrated samples that are a mixture of different zones within the screened interval (Pickens et al., 1978). Nesting wells or piezometers with short screens can be used to obtain samples from different depths, however this approach requires many bore holes and additional expense. The construction of a multi-level sampler allows for sampling of groundwater at closely spaced intervals in a vertical direction from a single bore hole. The MLS device used in this study is a slight modification of wells used in previous groundwater studies (LeBlanc et al., 1991; Boggs et al., 1988; Pickens et al., 1978).

MLS wells were constructed using 1.9 cm OD PVC pipe as the housing to which 0.6 cm OD polypropylene tubing was attached. For this study, 3–7 polypropylene tubes were attached to the outside of a 1.5–3 m section of PVC pipe by plastic cable ties. The ends of the sampling tubes attached to the pipe were wrapped twice with 202 mm Nytex mesh and spaced 40 cm apart. Enough tubing was left above the PVC pipe for easy access and sampling (~0.5 m). Upon installation of the well, the PVC pipe was filled with material removed from the borehole and then capped. Sample depths were identified at the top of each piece of tubing.

MLS sampling wells were installed using a hand auger with a 7.5-cm hollow barrel. To prevent the hole from back filling during construction, a 10-cm PVC casing (outer-casing) was inserted into the hole and moved downward as the hole was dug deeper. Once the clay layer was reached, the well was inserted into the hole, contained by the outer-casing. The outer-casing was then removed from the hole, allowing the aquifer materials to collapse around the sampler, isolating sampling points of the MLS at each level in the borehole. Additional soil material, originally removed from the hole, was back filled to complete the well as necessary. Wells were typically cut flush to the ground and covered with a removable 15 cm plastic cover.

2.4. Injections

Two tracer experiments were conducted at the Maxville site and one at the Brook Point site (Table 2). The injection solution was added by gravity feed into a pipe which was down stream of the septic tank and just upstream of the drainfield. This avoided the dilution of the injection slug in the septic tank. Prior to each injection, background samples were collected from the well field.

The Maxville site was first injected on December 3, 1999 with a solution consisting of 500 g of fluorescein dye dissolved into approximately 160 l of tap water which was bubbled with 99.8% pure SF_6 (Scott Specialty Gases) for at least 40 min. The injection slug was added to the drainfield over a 30-min period. An additional 20–30 l of tap water was used to rinse the container and added to assure that the injection solution was flushed from the distribution pipes into the drainfield. These same techniques were repeated at the Brooke Point site on December 20, 1999. The SF_6 concentration at approximately the midpoint of the injection period was 146 ± 9 μM, $n = 3$ at the Maxville site and 153 ± 3 μM, $n = 3$ at the Brooke Point site.

The chemical and viral tracers were combined in a second experiment at the Maxville site commencing on September 19, 2000. The injection slug consisted of a total of 1.29×10^{14} PFUs of PRD-1 and 250 ml of Rhodamine WT in approximately 160 l of onsite tap water, bubbled with 99.8% pure SF_6 (Scott Specialty Gases) for at least 40 min. In order to more closely mimic the dosing rate of a typical drainfield, the injection time was increased to two hours. To compensate for degassing, the SF_6 was allowed to bubble gently with the drum lid loosely closed during

the extended injection period. The SF_6 concentration was 77 ± 2 μM, $n = 2$ 30 min into the injection period and 109 ± 3 μM, $n = 2$ after 1.5 h.

2.5. Sample collection

Viral samples were collected from multi-level and slotted wells using either a 60-ml polypropylene syringe affixed with silicone tubing or a peristaltic pump. Sampling syringes and tubes were sterilized by autoclaving prior to each sampling and changed for each well. Samples were transported on ice to the laboratory facility for processing within 16 h.

Sulfur hexafluoride samples were collected directly from individual sample depths on each MLS well and slotted wells in 30-ml serum vials using a peristaltic pump. After purging the tubing or slotted well, a sample was pumped into a serum vial and allowed to overflow for three bottle volumes. The vial was then sealed with a rubber septa and a crimp cap. Since a small bubble is often present, the samples were stored on their sides until they could be extracted and analyzed, preventing loss of SF_6 in the bubble through the septa. Fluorescent dye samples were also collected with a peristaltic pump and stored in 100-ml amber polycarbonate containers.

2.6. Sampling frequency and duration

The first experiment at the Maxville site spanned 137 days with samples taken on days 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 26, 34, 40, 50, 55, 61, 76, 90, 110, 137. The second experiment at Maxville lasted 107 days with sampling events on days 0, 1, 2, 3, 6, 7, 10, 12, 16, 21, 27, 35, 44, 56, 69, 79, and 107. The experiment at the Brook Point site lasted 122 days with samples taken on days 0, 3, 7, 10, 14, 18, 25, 35, 37, 43, 57, 66, 80, 92, and 122. Samples for SF_6, the fluorescent dyes, and PRD-1 were taken consecutively within a 10-min period on each sampling day.

2.7. Sulfur hexafluoride sample analysis

Sulfur hexafluoride samples were extracted as described by Dillon et al. (1999). A small head space (typically 4 ml) of ultra-high purity nitrogen was added to the samples using a syringe. Simultaneously, a volume of water from the sample had to be removed and discarded to allow room for the head space. The serum vials were slightly over-pressurized with 1 cc of nitrogen to allow several injection volumes (100 μl or less) for the gas chromatograph (GC) to pull from each sample. After shaking for at least 2 min, this method extracts 95 + % of the SF_6 from a water sample (Dillon et al., 1999). The lower limit of this technique is 0.01 pM (10^{-14} mol/l).

Samples were analyzed with a Shimadzu model 8A gas chromatograph equipped with an electron capture detector. The gas chromatograph contained a stainless steel column (180 cm × 0.1 cm I.D.) packed with molecular sieve 5A (80/100 mesh). Ultra-high purity nitrogen was used as a carrier with a flow of 25 ml/min. Column and detector temperatures were set at 90 and 220 °C, respectively.

Head space concentrations, C, in ppmv (parts per million by volume, = μl/l) of SF_6 were determined by reference to a 1.04 ppmv standard (Scott Specialty Gases). The standard was run at the beginning of each day, after every 30 sample injections, and at the end of the day. Head space concentrations were converted to dissolved concentrations in μM as shown below:

$$C(\mu M) = (ml/l)/(R((1\ atm)/(mol\ K)) \times T(K) \times E$$

where R is the gas constant from the ideal gas law ($PV = nRT$), and T is temperature in degrees K. The parameter E is the extraction efficiency, 95% (Dillon et al., 1999).

Replicates were collected for at least 10% of the samples. In addition, duplicate injections were run on the gas chromatograph every tenth injection. Precision between replicate samples and duplicate injections were usually better than 10%.

2.8. Fluorescent dye analysis

The fluorescein and rhodamine samples were analyzed using a Turner Designs TD-700 Fluorometer, which provides exact concentrations after calibration. For fluorescein, the fluorometer used the 10-089 blue mercury vapor lamp, 10-105 excitation filter (486 nm), and the 10-109R-C emission filter (510–700 nm), as specified by the manufacturer. During rhodamine analysis, the fluorometer used the10-046 clear quartz lamp, 10-103 excitation filter (550 nm), and 10-052R emission filter (>570 nm), as specified by manufacturer. The fluorometer

was initially calibrated using fluorescein and rhodamine standards made in the laboratory. The lower limit of detection was 0.01 ppm and precision between replicate samples was greater than 10% and the reproducibility of duplicate analysis was greater than 5%.

2.9. PRD-1 analysis

PRD-1 samples were analyzed by plaque assay using a soft agar overlay technique with *Salmonella typhimurium* ATCC 19585 as a host. Two ml of sample were added to test tubes containing 3 ml of melted 1% TSB (trypticase soy broth) top agar (48 °C) and 1 ml of a 3 h culture of *S. typhimurium*, then poured onto solid TSA (tryptic soy agar) 1.5% agar plates. Five replicates of each sample were done for a total of 10 ml sample analyzed. The plates were incubated for 24 h at 37 °C. Plaques, areas where the viruses had grown and lysed the bacterial lawn, were then enumerated. Plaque forming units (PFUs) per 100 ml were calculated (Standard Methods for Examination of Water and Wastewater, APHA, 1992). When necessary, samples were serially diluted in sterile 1 × PBS (phosphate buffered saline) to obtain readable plates and assayed in duplicate.

3. Results

Most tracer studies calculate velocities from the time it takes a tracer to move from the point of injection to wells down gradient. Since the tracers were injected into the pipe where the sewage effluent enters the drainfield, velocities calculated in this manner include the residence time of the effluent in the drainfield. The first row of wells, at both sites, was on the edge of the top of the drain mound closest to the SIA (mound well, Fig. 2). Flow velocities of the injection slug within the drain mound are calculated from the injection point and time to these wells. The mound wells also provide a reference point for the effluent leaving the mound. The second row of wells was located at the toe or bottom of the mound and velocities calculated from row one (or the injection point) to these wells are representative of the flow of effluent from the elevated mound to grade. The transport velocity within the mound, and also exiting

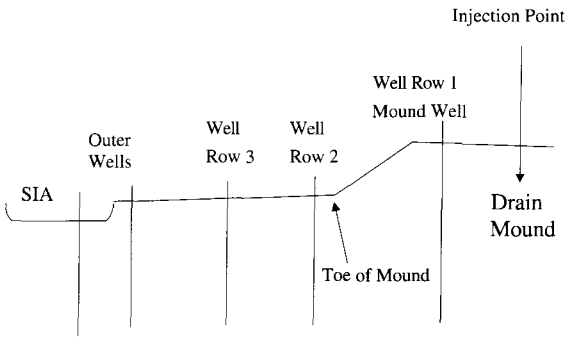

Fig. 2. Cross section schematic of the well field illustrating the different reference points for velocity calculations. Velocities calculated using the Injection Point and the mound wells in row 1 as reference points, are influenced by the drain mound and the change in elevation from the mound to grade. Velocities calculated from wells in rows 2 and 3 to the outer wells are least affected by the drain mound.

the mound, may be influenced by the fill materials of the mound and changes in soil characteristics made during drainfield installation. A third row of wells was installed further from the mound and several outer wells closer to and within the SIA. Velocities from the second row to the third row and outer wells represent the flow through soils not disturbed by the mound system (Fig. 2). Flow velocities calculated between wells in row 2 and those further from the mound yield results most representative of ground water movement between the drainfield mound and SIA. The primary direction of groundwater flow is indicated by the location of wells with multiple observations of relatively high concentrations of tracer.

Estimates of flow velocity between the same two wells can be calculated several different ways depending on which time references are used. For example, velocities can be calculated using various combinations of the initial observation and observed peak concentration of a tracer. The most appropriate time reference to calculate a velocity is based on the mean arrival time of the tracer. However, the sampling regime during this study was not frequent enough to confidently calculate the mean arrival time of the tracers. Therefore the observed concentration peak was assumed to better represent the passage of the bulk of the injection slug than the initial observation. Velocities calculated using the times of observed peak concentrations in both wells are preferable to velocities involving an initial observation in either well. In

several wells the tracer was observed only once, so the initial observation was also considered the peak concentration. The most reliable velocities are obtained from wells with multiple observations of tracer.

By applying a series of consistent preferences to a set of data, the velocities that are most representative of the movement of groundwater can be determined. Velocities were first grouped by location of the reference wells in the following order of preference: both wells at grade, well on mound to well at grade, and injection point to wells. Within these groupings, velocities calculated from peak to peak are preferred over velocities using an initial observation at either well.

3.1. SF_6 and fluorescein experiment Maxville, Duval County Winter/Spring 1999–2000

The drainfield was injected with SF_6 and fluorescein on December 3, 1999. SF_6 was detected within 6 h at the three mound wells, DsM1, DsM2, and DsM3 (first peak at 1.8 m, Fig. 3). At this site, the injection point was only 1.5 m away from DsM2 and 3 m from DsM1 and DsM3, with the drainfield extending in the opposite direction from the SIA (Fig. 1). During the initial stages of the injection, a small portion of the injection solution was observed pooling beneath the pipe to which we were adding the tracer. This pipe led from the septic tank to the drainfield distribution pipes. To remedy this situation, the injection rate was reduced resulting in the majority of the injection slug entering the distribution pipes of the drainfield. However, this small amount of leaked injection slug entered the water table very near the injection point and this input likely resulted in the observations within 6 h. SF_6 concentrations observed after day one are from the injection slug that did enter the distribution pipes and drainfield.

The largest concentrations of SF_6 were observed in well DsM2, with an observed peak (7.87 pM) occurring 10 days after injection (Fig. 3). Smaller

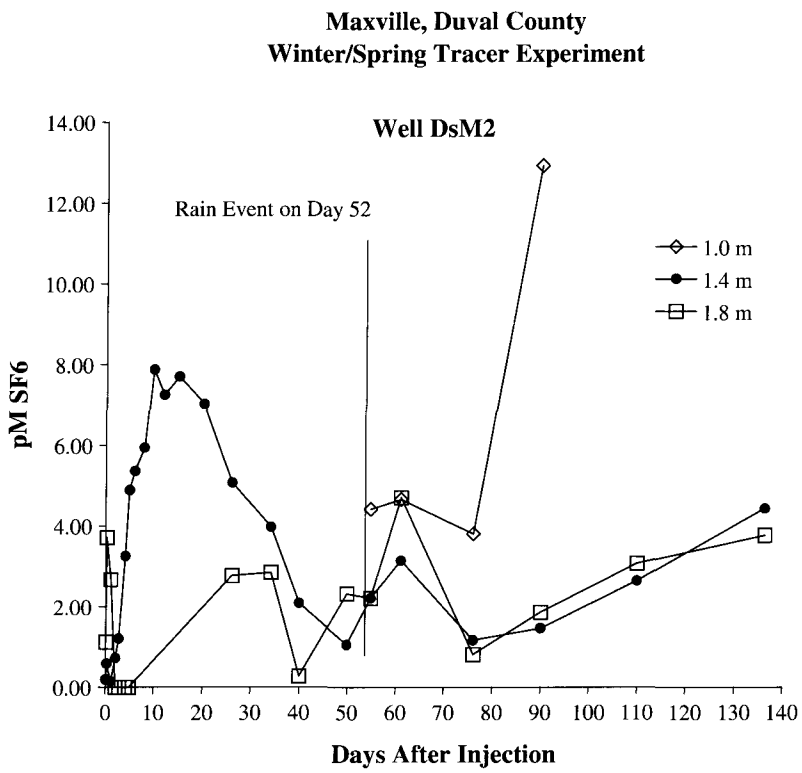

Fig. 3. Sulfur hexafluoride data for winter/spring tracer experiment at the Maxville site, injected on December 3, 1999. A peak was observed on day 10 at well DsM2, resulting in a velocity of 0.15 m/d calculated from the injection point and time. Rainfall on day 52 caused the water level to rise so that the 1.0 m depth could be sampled.

Table 3
Days after injection and concentrations of the SF_6 and fluorescein peak observations from the December 3, 1999 tracer experiment at Maxville, Duval County. Distance is relative to the injection point

	Distance (m)	SF_6 peak (days)	SF_6 peak (pM)	Fl peak (days)	Fl peak (ppm)
Wells on mound					
DsM1	3	12	0.9	90	0.5
DsM2	1.5	10	7.9	15	36.3
DsM3	3	34	1.0	110	51.4
Wells at toe of mound					
DsM4	8.5	50	0.8 ± 0.4	–	
DsM5	5.8	55	0.9	110	30.8
DsM6	5.7	50	0.6 ± 0.1	110	10.1
DsM7	8.4	55	1.1	–	
DsS1	5.4	26	5.8 ± 0.3	110	6.3
Third row, wells away from mound					
DsM8	12.7	55	0.7	–	
DsM9	10.0	50	0.4	–	
DsM10	9.0	50	0.7 ± 0.8	–	
DsM11	10.0	50	0.4	–	
DsM12	13.1	50	0.4	–	
DsS2	10.3	26	3.5 ± 0.4	–	
DsS3	8.2	26	2.8 ± 0.1	–	
DsS4	9.7	26	3.2 ± 0.3	–	
Outer wells, on edge of SIA					
DsM15	13.3	50	0.2	–	
DsM16	13.7	50	2.0 ± 0.0	–	
DsM17	14.1	61	0.1	–	
DsM18	13.1	50	0.2	–	
DsM26	15.5	61	0.2	–	
DsS6	11.4	50	0.3 ± 0.2	–	

concentrations were observed at DsM1 and DsM3, but DsM2 was the only well that continuously contained SF_6 and the peak concentration was significantly larger than other wells on or off the mound (Table 3). This indicates the bulk of the tracer flowed past DsM2 as it exited the mound.

The first appearance of SF_6 off the mound occurred on December 29, 1999, 26 days after injection. Although smaller concentrations were observed afterwards, the first observations of SF_6 were also concentration peaks in the slotted wells, DsS1, DsS2, DsS3 and DsS4, and trace amounts were observed (>0.1 pM) in DsM6 and DsM8 (Table 3). On day 50, the tracer appeared throughout the well array, except in wells DsM17, DsM26 and wells within the SIA. Peak concentrations were observed at wells DsM4, DsM6, DsM9, DsM10, DsM11, DsM12, DsM16 and DsM18. A large rain event occurred on day 52, and SF_6 was observed on day 55 in wells DsM5, DsM7, and DsM8. On day 61, SF_6 first appeared in wells DsM17 and DsM26 (Table 3). The tracer was not observed at the remaining outer wells in the SIA, DsM25, DsS5, DsS6, DsS7, DsS8, and DsS9 during the experiment. SF_6 was not found in any wells off the mound during the last four sampling events on days 76, 90, 110 and 137.

Besides the wells on the mound (DsM1-3) and those at the toe of the mound (DsM4-7), the only MLS wells with concentrations greater than 0.5 pM were DsM8, DsM10 and DsM16. The largest SF_6 concentrations off the mound were observed at DsM16 (1.98 ± 0.01 pM), directly in line with DsM2 and the SIA, indicating the bulk of the flow traveled due south from the drainfield. The data from the slotted wells is also consistent with this observation. Of the slotted wells, DsS1 and DsS3 had the highest concentrations and DsS6, also directly in line with DsM2, was the only outer slotted well in which SF_6 was observed. Velocities derived from wells along the DsM2 to DsM16 transect were most representative of the movement of the bulk of the tracer and thus effluent from the drainfield towards the SIA.

Transport velocities from the drain mound towards the SIA are reported from the injection point, not from well DsM2 due to an uncertainty in the arrival time of the tracer at DsM2 (mound to grade velocities, Table 4). The bulk of the tracer was found to pass by DsM2 on day 10, making this the logical choice for the reference well and time in velocity calculations. However, the SF_6 concentration on day 1 was also relatively large (3.7 pM), making the time reference at this well uncertain. Since the injection point is only 1.5 m from DsM2, velocities calculated from the injection point, 0.21 ± 0.08 m/d $n = 7$ are not significantly different than those calculated from DsM2 on day 1, 0.17 ± 0.07 m/d $n = 7$ or from day 10, 0.22 ± 0.11 m/d $n = 7$.

For velocities of groundwater movement that do not include the mound, the observed peaks on Day 26 at DsS1 and DsS3 were the best reference points to calculate velocities to wells further from the mound DsM10, DsM16 and DsS6 (Table 4). Both reference points were used for grade-to-grade velocity calculations since DsS3 was further from the mound, yet

Table 4
Velocities calculated from the SF_6 and fluorescein results from the December 3, 1999 tracer experiment at Maxville, Duval County. All velocities are reported in units of m/d

	SF_6 peak	Fl peak
Velocities within the mound		
Injection point to DsM1	0.25	0.03
Injection point to DsM2	0.15	0.10
Injection point to DsM3	0.09	0.03
Average	0.16 ± 0.08	0.05 ± 0.04
Velocities mound to grade		
Injection point to DsM5	0.11	0.05
Injection point to DsM6	0.11	0.05
Injection point to DsS1	0.21	0.05
Injection point to DsM10	0.18	
Injection point to DsS3	0.31	
Injection point to DsS6	0.26	
Injection point to DsM16	0.27	
Average	0.21 ± 0.08	0.05 ± 0.00
Velocities at grade		
DsS1 to DsM10	0.16	
DsS1 to DsS6	0.32	
DsS1 to DsM16	0.37	
DsS3 to DsM10	0.04*	
DsS3 to DsS6	0.20	
DsS3 to DsM16	0.25	
Average	0.22 ± 0.12	
Average (without *)	0.26 ± 0.09	

the concentration at DsS1 (5.80 ± 0.34 pM) was larger than at DsS3 (2.80 ± 0.06 pM). DsS2 and DsS4 were not chosen as reference points, since they are on either side of the DsM2 to DsM16 transect. The transport velocity of 0.26 ± 0.09 m/d $n = 5$ is the best estimate from the SF_6 data and does not include the velocity form DsS3 to DsM10. The distance between these wells is only 0.9 m, and the velocity calculated, 0.04 m/d, may have been skewed by the short distance and low sampling frequency. Alternatively, the flow may not have been aligned between DsS3 and DsM10 and the measured SF_6 represents different potions of the injection solution.

The movement of fluorescein in the well field was retarded with respect to SF_6. Even after 137 days, fluorescein was observed in only six wells, DsM1, DsM2, DsM3, DsM5, DsM6, and DsS1 and in each case dye peaks lagged behind SF_6 peaks (Table 3, Fig. 4). Since fluorescein did not appear past the toe of the mound, only transport velocities within the mound and exiting from the mound can be calculated. Fluorescein transport velocities are reported in Table 4 and were approximately 25% of the SF_6 velocities.

3.2. Sulfur hexafluoride, PRD-1 and rhodamine WT experiment Fall 2000

A second tracer experiment was conducted at the Duval County-south site. The drainfield was injected with a solution containing SF_6, rhodamine WT, and PRD-1 on September 19, 2000. The continuing presence of fluorescein in the well field from the previous experiment necessitated the use of the rhodamine dye. The injection slug was added over a 2-h period in contrast to the 30-min injection time during the December 3, 1999 experiment. By spreading the injection time over 2 h, we avoided the initial pooling of the tracer under the injection pipe connecting the tank and the drainfield, which occurred in the first experiment. The site was very wet on the day of injection, with standing water over wells DsM4 and DsM19.

Background samples prior to this injection contained SF_6, in addition to fluorescein, presumably from the previous experiment. The rains prior to this injection could have mobilized a portion of the SF_6/fluorescein injection slug possibly held within the drainfield. SF_6 was observed in well DsM2 4 months after the December 3, 1999 injection, indicating SF_6 could reside in drainfield mounds for long periods of time. SF_6 was observed 3 days after injection in all the wells, including the slotted wells in the SIA (DsS5, DsS6, DsS7, DsS8, and DsS9), lending further support to the hypothesis that a pool of the prior injection slug was mobilized. A possible explanation for the persistence of SF_6 in the drainfield was that it may have been held in an oil phase in greasy materials of the drainfield mound. SF_6 is known to partition from water to oily phases and materials in the drainfield mound may provide conditions for this to occur. Velocities could not be calculated for the SF_6 observations during the first 3 days of the experiment due to the uncertainty in whether the observed tracer originated from the injection solution or was already in the system prior to the start of the experiment. After 3 days, concentrations of SF_6 generally decreased and later

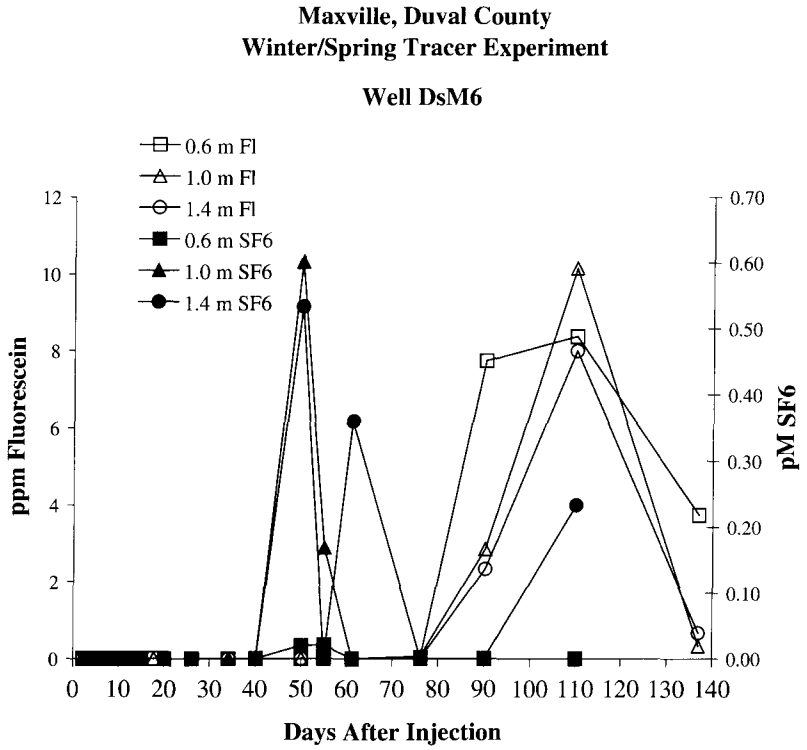

Fig. 4. Sulfur hexafluoride (closed symbols) and fluorescein (open symbols) data for well DsM6 during the winter/spring tracer experiment at Maxville, Duval County. The drainfield was injected on December 3, 1999. The fluorescein peak occurred 60 days after the SF_6 peak.

larger concentrations were observed after a second rain event on days 9 and 10 of the experiment.

On day 12, two days after a large rain event on September 29–30, 2000, the tops of wells DsM4, DsM19, and DsM20 were all under water. On day 21, SF_6 was observed throughout the well field at: well DsM2 on the mound; DsM5, DsM6, DsS1 in row 2, directly in front of the mound; and in row three at wells DsM10 and DsS3. All SF_6 observations on day 21 were several times larger than the concentrations observed on day 3 (Fig. 5) and are assumed to be from the September 19, 2000 injection and not residual SF_6 from the previous experiment.

On Day 27, significant SF_6 concentrations were observed at DsS2, DsS4, and DsS6. Only trace amounts of SF_6 (>0.2 pM) were observed at the wells outside the DsM2 to DsS6 transect: DsM4, DsM7, DsM8, DsM9, DsM11, and DsM12. However, SF_6 was observed at DsM9 (0.47 pM) on day 79, which occurred after a rise in the water table depth on day 69. As in the previous experiment, the highest concentrations of SF_6 were found in wells in line with DsM2 and DsS6, with smaller concentrations found in wells to either side of this transect.

The presence of SF_6 in the drainfield prior to this experiment complicates velocity calculations by placing the time reference in question. Fortunately, SF_6 concentrations occurring in the main plume were several times larger than background levels, indicating the observed SF_6 was from the latest injection. Further support is given by the lack of additional observations of SF_6s at wells DsM5, DsM6. DsS1, DsM10 and DsS3 during the remainder of the 107-day experiment (Fig. 5). Since the peak concentration was observed in wells on and off the mound on Day 21, the injection point and time is used for velocity calculations (Table 5).

The first detection of PRD-1 occurred on day 21 in wells DsM1, DsM2, DsM6 and DsM10. All of these wells also had coinciding peak SF_6 concentrations occurring on day 21. PRD-1 was first observed in the slotted wells DsS1 and DsS4 on day 35 and in the MLS

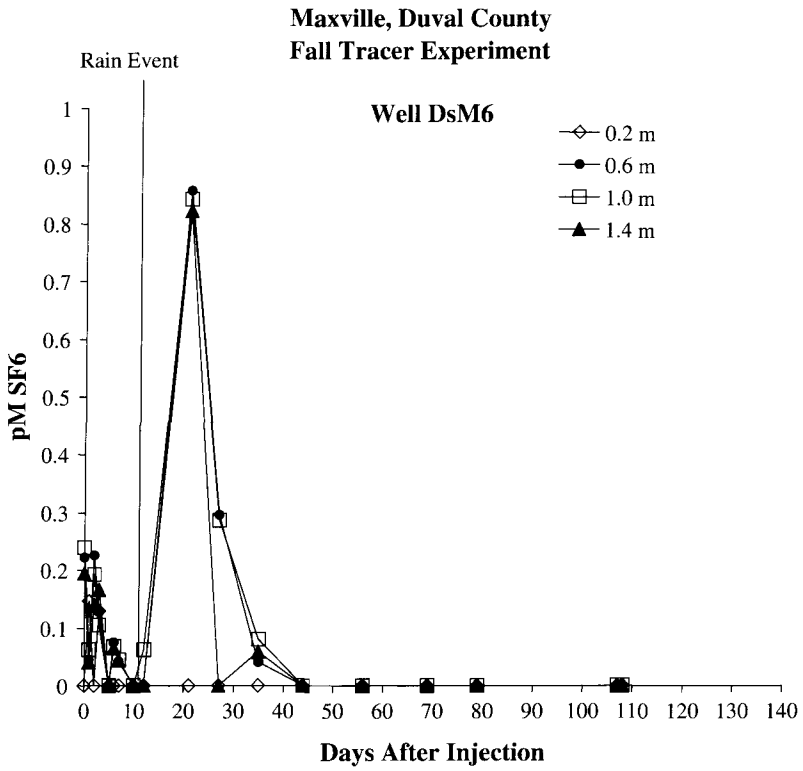

Fig. 5. Sulfur hexafluoride data for the fall tracer experiment at Maxville, Duval County injected on September 19, 2000. The site was wet during injection with standing water at the toe of the drain mound and a rain event occurred on day 10 causing similar conditions. A peak was observed at well DsM6 on day 21, resulting in a velocity of 0.27 m/d calculated from injection point and time. See text for explanation of initial observations in the first 3 days.

wells DsM9, DsM11, DsM16, DsM17, and DsM18 on day 44. The first appearance of PRD-1 in well DsM15 was on day 79 of the study (Table 5). PRD-1 velocities were calculated using the initial observance of the tracer. PRD-1 first appeared both on the mound in wells DsM1 and DsM2 and off the mound in DsM6 and DsM10 on the same day, therefore flow velocities were calculated using the injection point as a reference point as was done for the SF_6 data (Table 6). Fig. 1 shows the Maxville site with days until first appearance of PRD-1 tracer in respective wells.

Rhodamine was only observed on the mound in wells DsM2 and DsM3 during the experiment. The peak concentration at DsM2 lagged behind the SF_6 peak by six days over the 1.5 m distance from the injection point (Fig. 6). The observed peak at DsM2 (722 ppb) is over two orders of magnitude greater than the detection limits (1 ppb), indicating the lack of further observations of rhodamine in the well field is not due to dilution but to retention in the drainfield and soils. These results indicate rhodamine flow is much slower than both SF_6 and fluorescein in this system.

3.3. SF_6 and fluorescein experiment Alachua County Winter/Spring 1999–2000

The drainfield at the Alachua county site was injected with SF_6 and fluorescein on December 20, 1999. Following a rain event on day 35 in otherwise drought conditions, SF_6 was observed throughout the well field. On days 37 and 43, peaks were observed in all the MLS wells and the slotted wells AS1-4, resulting in a velocity of 0.42 ± 0.10 m/d $n = 20$ calculated from the injection point. In contrast, fluorescein was only observed in one well off the mound, at AS2 located 13.25 m from the injection

Table 5
Days after injection and concentrations of the SF_6 peak observations and PRD-1 initial appearance from the September 19, 2000 tracer experiment at Maxville, Duval County. All of the Peaks occurred after the rain event on September 29–30, 2000. Distance to wells is relative to the injection point

	Distance (m)	SF_6 peak (days)	SF_6 peak (pM)	PRD-1 initial (days)
Wells on mound				
DsM1	3	21	0.8 ± 0.0	21
DsM2	1.5	21	2.1 ± 0.1	21
DsM3	3	21	0.8 ± 0.2	–
Wells at toe of mound				
DsM4	8.5	27	0.3 ± 0.1	–
DsM5	5.8	21	0.8 ± 0.0	–
DsM6	5.7	21	0.9 ± 0.1	21
DsS1	5.4	21	1.0 ± 0.1	35
Third row, wells away from mound				
DsM8	12.7	21	0.8 ± 0.0	–
DsM9	10.0	79	0.5	44
DsM10	9.0	21	1.1 ± 0.0	21
DsM11	10.0	–		44
DsS2	10.3	27	0.4 ± 0.1	–
DsS3	8.2	21	0.7 ± 0.1	–
DsS4	9.7	27	0.3	35
Outer wells, on edge of SIA				
DsM15	13.3	–		79
DsM16	13.7	–		44
DsM17	14.1	–		44
DsM18	13.1	–		44
DsS6	11.4	27	0.3	44

Table 6
Velocities calculated from the September 19, 2000 tracer experiment at Maxville, Duval County SF_6 and PRD-1 results. All velocities are reported in m/d. Observed concentration peaks in wells off the mound all occurred after the rain event September 29–30, 2000

	SF_6 peak	PRD-1 initial
Velocities within the mound		
Injection point to DsM1	0.14	0.15
Injection point to DsM2	0.05	0.07
Injection point to DsM3	0.14	
	0.11 ± 0.05	0.11 ± 0.05
Velocities exiting the mound		
Injection point to grade	SF_6 peak	PRD-1
Injection point to DsM5	0.28	
Injection point to DsM6	0.28	0.28
Injection point to DsM9	0.28	0.23
Injection point to DsM10	0.43	0.43
Injection point to DsM11		0.23
Injection point to DsM15		0.17
Injection point to DsM16		0.32
Injection point to DsM17		0.32
Injection point to DsM18		0.30
Injection point to DsS1	0.26	0.16
Injection point to DsS2	0.38	
Injection point to DsS3	0.39	
Injection point to DsS4	0.36	0.28
Injection point to DsS6	0.48	0.30
Average	0.35 ± 0.08	0.27 ± 0.08
Average of main plume	0.35 ± 0.09	0.30 ± 0.10

point at the toe of the mound. The fluorescein peak at AS2 occurred 23 days after the SF_6 peak, yielding a velocity of 0.20-m/d, which is two thirds of the SF_6 velocity at this well, 0.31 m/d. The lack of fluorescein observations in any other well is strong evidence that fluorescein is retained by the soils in the mound and well field.

4. Discussion

4.1. SF_6 and fluorescent dyes

Both fluorescent dyes were retarded in the onsite systems with respect to SF_6. In each experiment the SF_6 traveled further into the well field and was observed in many more wells than either dye. At both sites, fluorescein was not observed past the toe of the mound, while SF_6 was observed throughout the well array. These fluorescein peaks lagged behind SF_6 peaks in every instance. At the Maxville site, the fluorescein peak occurred 5 days after the SF_6 peak at mound well DsM2. Approximately 4 m further from the injection point, at the toe of the mound, the observed separation of the two tracer peaks was 55 days at DsM5, 60 days at DsM6 (Fig. 4), and 84 days at DsM1. The SF_6 velocity of 0.11 ± 0.00 m/d from these wells is twice that of the fluorescein velocity 0.05 ± 0.00 m/d (Table 4). Rhodamine WT was held preferentially to SF_6 by the mound at Maxville to an even greater degree, with the only observations of the dye at wells DsM2 and DsM3. Although the distance between the injection point and DsM2 was only 1.5 m, the rhodamine peak occurred 6 days after the SF_6 peak (Fig. 6). When observed, the dye concentrations were

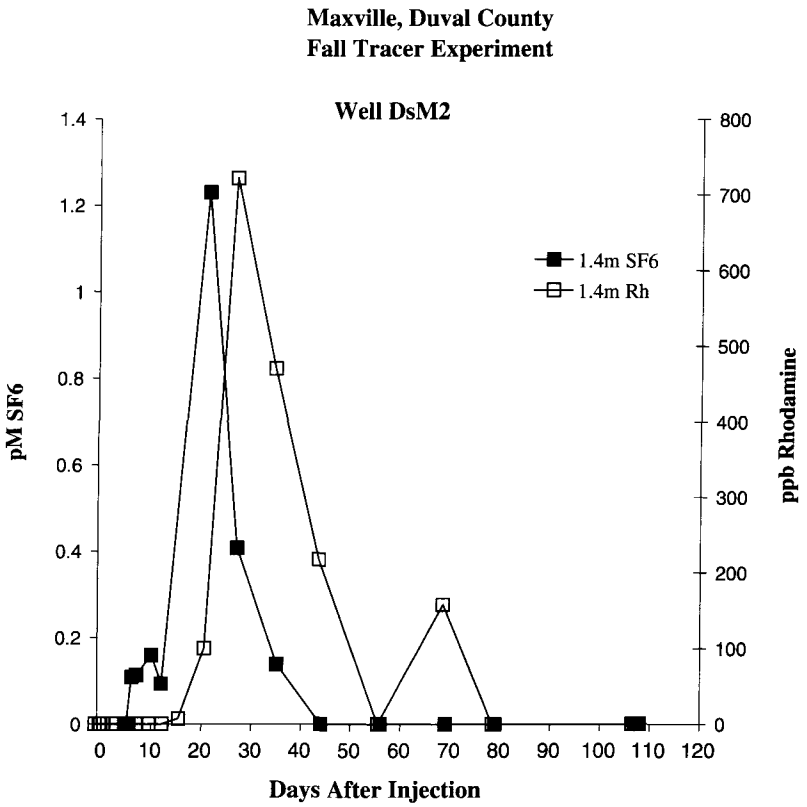

Fig. 6. Sulfur hexafluoride (closed symbols) and rhodamine WT (open symbols) data for well DsM2 during the fall tracer experiment at Maxville, Duval County. The drainfield was injected on September 19, 2000. The rhodamine WT peak occurred 6 days after the SF_6 peak over a distance of 1.5 m.

relatively large, indicating that dilution of the injection slug was not the reason for the lack of further observations. These results are consistent with results obtained by Sabatini and Austin (1991), who observed rhodamine WT being adsorbed to a greater extent than fluorescein in alluvial aquifer sands.

The Alachua winter/spring data also presents further evidence fluorescein is retarded compared to SF_6. The velocity from the fluorescein data, 0.20 m/d is approximately 50% of the SF_6 velocity 0.42 ± 0.10 m/d $n = 20$. Additionally, SF_6 was observed throughout the well field during the experiment, while fluorescein was only seen at AS2 and the five mound wells.

Although used frequently as a tracer in onsite systems, fluorescein is known to bind to organic matter in soils (Trudgill, 1987; Omoti and Wild, 1979; Smart and Laidlaw, 1977) and also alumina and carbonates (Kasnavia et al., 1998). At both study sites, SF_6 was found in more wells and further from than the injection point than fluorescein. The amount of separation between the SF_6 data and the fluorescein data increases with distance from the injection point, further supporting the hypothesis of its sorption to the soils. With respect to fluorescein, the soils in the well field are acting much as chromatography column. The velocities calculated from the fluorescein data should be considered low for transport of the groundwater, but may be representative of some component of the effluent with similar sorptive characteristics.

4.2. SF_6 and PRD-1

Caution must be used when comparing velocities obtained from chemical tracers and those from viral tracers. Since the PRD-1 tracer is used to model transport of enteric viruses, which can have a low infectious dose and inactivation of virus particles can

occur over time thereby affecting concentrations, velocities are calculated using the initial appearance of the tracer as the time reference rather than peak concentrations. This approach may yield slightly higher velocities. However, such viral transport velocities, particularly data from field tracer studies, along with risk assessment models have been used to address public health risks associated with waterborne viral infections (Rose and Yates, 1998). Often the first appearance of the viral tracer was the only appearance, simplifying the comparison. In contrast, velocities obtained from quantitative, chemical tracers are generally calculated using the tracer peak as the time reference. Velocity calculations from peak observations yield more reliable results than those using initial observations. The concentration peak represents the passage of a significant amount of the tracer, and the multiple data points used in determining the peak increase the level of confidence. As a dissolved gas, velocities from SF_6 represent the actual movement of the groundwater. Despite the differences in calculation methods, the velocities from the SF_6 data are similar to those from the PRD-1 data. At wells DsM6 and DsM10, the peak concentration of SF_6 occurred on the same day as the initial appearance of PRD-1. The total data set indicates the PRD-1 moves virtually at the same velocity, 0.27 ± 0.08 m/d $n = 11$, as the groundwater as indicated by the SF_6 velocity, 0.35 ± 0.08 m/d $n = 9$.

At both the Maxville and Alachua sites, SF_6 was observed throughout the well fields after large rain events. At the Maxville site, PRD-1 was also observed in many wells after the large rain event, suggesting the movement of PRD-1 tracer coincided with that of SF_6. However, it is possible that SF_6 may have an alternate transport mechanism that differs from PRD-1. We cannot rule out the possibility that the SF_6 may have been present in the vadose zone prior to the rain event, a possible scenario considering the loss due to volatilization during injection. After the rain event, the SF_6 may have been was either washed from the vadose zone into the groundwater by infiltration or the groundwater table rose to encounter the SF_6. It is also possible that any SF_6 entering the vadose zone would be lost to the atmosphere. While further research needs to be conducted to determine if SF_6 and PRD-1 do indeed have similar transport mechanisms, the results of our study are consistent with the hypothesis that they do.

4.3. Influence of mound on groundwater velocities

The first experiment at the Maxville site yielded similar values for flow velocities that include the drain mound (velocities mound to grade, Table 4) and those that are away from the mound (velocities at grade, Table 4). These results suggest that the drainfield mound is dominating the hydrology between the mound and SIA and that the groundwater flow velocities may be elevated by the presence of the system. During the drought conditions of the experiment, the water from the mound system created a gradient towards the dry SIA. Conditions during the second experiment at Maxville were wetter, especially during the initial two weeks, and the velocities were faster. Calculated from the injection point, the transport velocity was 0.21 ± 0.08 m/d for the first experiment and 0.35 ± 0.08 m/d during the wetter second experiment.

Significant degassing of SF_6 was observed during all three experiments. The drainfields were injected with solutions containing approximately 100 μM SF_6 yet the highest concentrations measured in the wells were on the order of 10 pM. One would expect degassing of SF_6 in the elevated mound systems and this effect was aggravated by the drought conditions and depressed water table during the experiments. Despite this degassing, sufficient SF_6 entered the groundwater for successful flow measurements. Extending the injection time from 30 min to 2 h did not significantly effect the measured concentrations between the two tracer experiments at the Maxville site.

5. Conclusion

Results from this study indicate that fluorescein and rhodamine WT do not move with the groundwater, but are retained by the drainfield and soils of mounded systems adjacent to seasonally inundated wetlands. Although a large portion of SF_6 in the tracer solution is thought to have degassed prior to entering the groundwater, SF_6 appeared to be a successful tracer of sewage effluent from mounded systems

towards depressional wetlands. Comparisons to the viral tracer PRD-1 are consistent with the hypothesis that velocities from SF_6 tracer studies may be used to simulate viral movement. The apparently similar velocities obtained from SF_6 and PRD-1 tracers suggest that the differences observed in previous studies in the Florida Keys are due to environmental site and temporal variability.

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