ASSESSING THE POTENTIAL EFFECTS OF FUNGICIDES ON NONTARGET GUT FUNGI (TRICHOMYCETES) AND THEIR ASSOCIATED LARVAL BLACK FLY HOSTS

SUPPORTING INFORMATION

Pesticide analysis, detection limits and quality control.

Black fly extraction. Thawed composite black fly larvae samples (0.12 to 2.5 g) from each site were analyzed for a suite of 12 pesticides. Prior to extraction tissue samples were spiked with trifluralin-d_{10}, ring-^{13}C-*p,p'*-DDE, and phenoxy-^{13}C-*cis*-permethrin (Cambridge Isotopes, Andover, Massachusetts) as recovery surrogates. Composite larvae samples were homogenized with Na_2SO_4 and extracted using a sonic water bath at 30°C for 25 min. Samples were extracted two times with dichloromethane (DCM). Extracts were reduced to 1 ml using a Turbo Vap II (Zymark) operating at 25°C with high purity (>99.99 %) N_2. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance. Due to the small sample mass and non detectable amounts of lipid, no clean-up was necessary. Samples were exchanged to ethyl acetate, further reduced to 200 µl, and acenaphthene-d_{10} was added to each sample prior to analysis as an internal standard.

Instrumental analysis. The surface water extracts (1 µl injection volume) were analyzed on an Agilent 5975 gas chromatograph (GC)/electron ionization mass spectrometer (EI-MS) (Folsom, CA, USA). Analyte separation on the GC was achieved using a 30 m x 0.25 mm i.d., 0.25 µm DB-5ms fused silica column (Agilent Technologies, Folsom, California) with helium as the carrier gas. The temperature of the splitless injector was held constant at 275°C. The

temperature program for all herbicides and insecticides was as follows: 80°C (hold 0.5 min), increase to 120°C at 10°C /min, increase to 200°C at 3°C/min (hold 5 min), followed by a third increase to 219°C at 3°C/min, and a final increase to 300°C at 10°C/min (hold 10 min). The temperature program for the fungicides was as follows: 80°C (hold 0.5 min), increase to 180°C at 10°C/min, increase to 220°C at 5°C/min (hold 1 min), increase to 280°C at 4°C /min (hold 1 min), and, finally, increase to 300°C at 10°C/min (hold 10 min). The transfer line, quadrupole, and source temperatures were 280°C, 150°C, and 230°C, respectively. Data for all pesticides were collected in selective ion monitoring mode (SIM), with each compound having one quantifier ion and 1-2 qualifier ions (Table S1).

Black fly tissue extracts (1 µl injection volume) were analyzed using an Agilent 7890 gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies, Folsom, California, USA) operating in multiple reaction monitoring (MRM) mode. Analyte separation for larval black fly tissue on the GC was achieved using a 30 m x 0.25 mm i.d., 0.25 µm DB-5ms fused silica column (Agilent Technologies, Folsom, California). The temperature of the splitless injector was held constant at 275°C. The temperature program was as follows: 80°C (hold 1 min), increase to 220°C at 20°C/min (hold 1 min), and a final increase to 300°C at 20°C/min (hold 5 min). The transfer line and electron ionization source temperatures were 250°C. An electron ionization energy of 70 eV was used with a filament-multiplier delay of 5 min. The filament current was 35 µA, N_2 was used as the collision gas with a flow of 1.5 ml/min, and the He flow was 2.5 ml/min. The temperatures of the quadrupoles were 150°C and 300°C. The detector voltage was automatically set by the instrument after automated MS/MS tuning, which was typically 1300 V. A full autotune of the mass spectrometer using the default

parameters was performed prior to each sequence. Agilent MassHunter software was used for instrument control and data acquisition/processing.

The final MRM acquisition method consisted of 2 ion transitions at the experimentally optimized collision energy (CE) for each analyte and a dwell time of 2.5 ms was set for all transitions (inter dwell delay of 1 ms). The "wide" MS resolution setting of 1.2 amu full width at half maximum was entered into the MRM method for all transitions. Information on MRM transitions and CE for each compound can be found in Table S1.

Instrument calibrations were achieved using concentration standards that spanned the linear range of instrument response. Calibration curves were considered acceptable if the R^2 for each individual compound was greater than 0.995. The responses of the instrument were monitored every 6-8 samples with mid-level check standards. The instruments were considered to be stable if the recovery of the check standards fell within the range of 80-115% of the nominal standard concentration. If environmental sample concentrations fell outside the linear range of the instrument, the samples were diluted appropriately and re-analyzed.

Detection Limits

Surface water. Surface water method detection limits (MDLs) were previously validated for the majority of the pesticides (Hladik *et al*., 2008; Reilly *et al*., 2012b) using the EPA procedure described in 40 CFR Part 136 (EPA, 1992). Water samples used to determine MDLs for insecticides and herbicides were collected in 2005 from the Sacramento River at Miller Park and water samples for fungicide MDLs were collected in 2008 from the American River near the California State University Campus. MDLs for all compounds in water ranged from 0.9 to 10.5 ng/l and instrumental limits of detection (LOD) ranged from 0.5 to 1.0 ng/l (Table S1). Analytes

detected at concentrations greater than the instrumental LOD but less than the MDL were reported as estimates.

Larval tissue samples. Instrumental LOD were calculated for the 12 pesticides included in the method (Table S1). LODs ranged from 0.001 to 0.004 µg/g wet weight and were based on the lowest measurable calibration standard divided by an average wet mass of tissue (1 g).

Quality Assurance

Pesticide concentrations in water and black fly larvae were validated against a comprehensive set of performance based quality assurance/quality control (QA/QC) criteria including laboratory blanks, matrix spikes, and surrogate recovery.

Surface water. Eight laboratory blanks were processed to test the cleanliness of the laboratory procedures. No pesticides were detected in any of the blank samples. Ring-$^{13}C_3$-atrazine and diethyl-d_{10} diazinon were used as recovery surrogates to assess the efficiency of sample extraction. Percent recovery of surrogates for all samples analyzed (including QC samples) ranged from 73 to 118% with a mean (± standard deviation) of ring-$^{13}C_3$-atrazine and diethyl-d_{10} diazinon of 89 ± 9% and 93 ± 12%, respectively. Six samples were spiked in the laboratory with a suite of 90 pesticides and the percent recovery ranged from 78-110% with a median of 92%.

Larval tissue samples. Two laboratory blanks were processed with the 17 environmental tissue composite samples and no pesticides were detected in the blank samples. Trifluralin-d_{10} was used as a recovery surrogate and the percent recovery for all samples analyzed (including QA) ranged from 81 to 126% with a mean (± standard deviation) of 108 ± 12%. Four samples were spiked in the laboratory with the 12 pesticides and the percent recovery of the spiked samples ranged from 73 to 126% with a median of 97%.

Individual larval pesticide body burden concentrations.

Individual black fly larvae were composited in the field for pesticide analysis. Composite weights varied by site and date and ranged from 0.1 to 2.5 g wet weight with a median of 0.7 g (Table S2). Individual larval body burden concentrations were estimated by counting the number of black fly larvae in each sample. An estimate of the mass of the individual larvae was then calculated based on the number of larvae in the composite sample and the known composite mass. The estimated mass of the individual larvae ranged from 0.8 to 2.8 mg with a median of 1.6 mg and varied by site and date (Table S3). The mass of the individual larvae has the potential to vary within a composite depending on age and stage of development; therefore the calculation is only an estimate because it assumes that all larvae weigh the same within the composite. Larval instar stage of the collected samples for pesticide analysis was not determined in the field. Estimated pesticide concentrations in individual larvae concentrations in µg/g wet weight were calculated based on the concentration of the pesticide in the composite sample and the estimated individual larvae mass.

$$C_{ind} = (C_{comp} \times m_{comp})/(m_{ind}/1000)$$

The equation reads as follows: C_{ind} is the concentration of the pesticide in the individual black fly larvae (µg/g), C_{comp} is the concentration of the pesticide in the composite sample (µg/g), m_{comp} is the mass of the composite sample (g), and m_{ind} is the estimate mass of the individual larvae within the composite (mg).

LITERATURE CITED

Hladik, M. L., Smalling, K. L. & K. M. Kuivila, 2008. A Multi-Residue Method for the Analysis of Pesticides and Pesticide Degradates in Water Using HLB Solid-Phase Extraction and Gas Chromatography-Ion Trap Mass Spectrometry. *Bulletin of Environmental Contamination and Toxicology* 80:139-144.

Reilly, T. J., Smalling, K. L., Wilson, E. R., and W. Battaglin, 2012b. Dissolved Pesticides, Dissolved Organic Carbon, and Water-Quality Characteristics in Selected Idaho Streams, April–December 2010. U.S. Geological Survey Data Series 667, 17 p. http://pubs.usgs.gov/ds/667/

U.S. Environmental Protection Agency, 1992, Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11: Code of Federal Regulations 40, Protection of the Environment, CFR Part 136, Appendix B, p. 565–567

LIST OF TABLES

Table S1. Retention time, MRM conditions, qualifier and qualifier ions, average percent recovery of matrix spikes, and instrumental limits of detection (LOD) for pesticides analyzed in larval black fly tissue.

	MRM transitions						
	RT	**Quantifier**	**CE (V)**	**Qualifier**	**CE (V)**	**Average Recovery (%)**	**LOD (µg/g)**
s-ethyl dipropylthiocarbamate (EPTC)	6.06	189 > 86	10	189 > 128	10	103 (12)	0.001
Ethalfluralin	7.94	276 > 202	20	276 > 105	20	99 (11)	0.001
Trifluralin	8.02	306 > 264	14	264 > 160	20	97 (10)	0.001
Simazine	8.45	201 > 173	25	201 > 158	10	104 (10)	0.001
Atrazine	8.51	200 > 104	25	200 > 94	25	96 (10)	0.001
Metolachlor	10.12	162 > 132.1	30	162 > 91	30	101 (10)	0.001
Pendimethalin	10.57	252 > 162	16	252 > 191.1	14	103 (8)	0.001
Imazalil	11.23	173 > 109	38	173 > 73.9	38	79 (6)	0.002
Hexazinone	12.32	171 > 71	16	171 > 85	14	102 (12)	0.001
Pyraclostrobin	13.67	132 > 77	30	132 > 51	34	101 (20)	0.001
Boscalid	14.51	140 > 112.1	20	140 > 76	30	95 (13)	0.001
Azoxystrobin	16.29	344 > 329	10	344 > 156	34	91 (9)	0.004

CE: collision energy in volts.
RT: retention time

Table S2. Pesticide concentrations (ng/l) in surface water from the two reference (Cottonwood Creek and Dry Creek) and two agricultural (Wanstad Road Ditch and Sand Run Gulch) sites collected between April and December 2010.

Station Name	Date	Time	3,5-DCA	Alachlor	Atrazine	Azoxystrobin	Boscalid	Chlorothalonil	Chlorpyrifos	Clomazone	Diazinon	EPTC	Ethalfluralin	Fipronil	Hexazinone	Imazalil	Metolachlor	p,p'-DDD	Pendimethalin	Propiconazole	Pyrimethanil	Simazine	Tetraconazole	Trifluralin	Triflumizole
Cottonwood Creek	4/21/10	1525	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	5/13/10	1100	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	6/2/10	1300	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	6/22/10	1300	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	7/12/10	1200	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	8/2/10	930	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	8/23/10	900	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	14.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	9/15/10	940	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	10/4/10	1230	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	11/12/10	1230	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	12/6/10	1330	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dry Creek	6/2/10	945	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	6/22/10	1300	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	7/12/10	1200	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	8/2/10	930	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	8/23/10	900	nd	nd	21.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	142	nd	nd	nd
	9/15/10	940	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	10/4/10	1430	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	10/25/10	845	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	11/12/10	1030	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	12/6/10	950	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected

Table S2 cont. Pesticide concentrations (ng/l) in surface water from the two reference (Cottonwood Creek and Dry Creek) and two agricultural (Wanstad Road Ditch and Sand Run Gulch) sites collected between April and December 2010.

Station Name	Date	Time	3,5-DCA	Alachlor	Atrazine	Azoxystrobin	Boscalid	Chlorothalonil	Chlorpyrifos	Clomazone	Diazinon	EPTC	Ethalfluralin	Fipronil	Hexazinone	Imazalil	Metolachlor	p,p'-DDD	Pendimethalin	Propiconazole	Pyrimethanil	Simazine	Tetraconazole	Trifluralin	
Sand Run Gulch	4/13/10	1430	nd	nd	9.60	1.8	8.20	nd	nd	nd	nd	nd	nd	nd	771	205	21.6	nd	nd	nd	nd	10.6	nd	nd	n
	5/3/10	1320	nd	nd	5.80	2.2	10.2	nd	nd	nd	nd	nd	9.0	nd	96.6	nd	122	nd	34.2	nd	nd	nd	nd	nd	n
	5/24/10	1430	nd	nd	nd	1.4	4.42	nd	nd	nd	nd	23.3	nd	nd	137	nd	66.9	nd	28.5	nd	nd	nd	nd	nd	n
	6/16/10	1145	nd	nd	28.1	nd	nd	nd	nd	nd	nd	22.9	nd	nd	nd	nd	171	nd	38.9	nd	nd	nd	nd	nd	n
	7/6/10	1340	nd	nd	7.51	6.29	10.8	nd	nd	nd	nd	9.54	5.89	nd	16.9	nd	137	nd	45.9	nd	nd	nd	nd	1.2	n
	7/26/10	950	nd	nd	nd	11.4	16.0	nd	nd	nd	nd	nd	3.20	nd	nd	nd	78.4	nd	nd	nd	nd	nd	nd	2.0	n
	8/16/10	1200	nd	nd	nd	20.2	37.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	16.2	nd	nd	nd	nd	nd	nd	nd	n
	9/9/10	1135	nd	nd	4.31	12.5	14.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	19.6	nd	nd	nd	nd	nd	nd	nd	n
	9/29/10	930	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.0	nd	nd	nd	nd	nd	nd	nd	n
	10/18/10	945	nd	nd	nd	2.6	5.41	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.8	5.2	nd	4.8	nd	1
	11/8/10	920	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.40	nd	nd	nd	nd	nd	nd	nd	n
	11/29/10	1100	nd	nd	6.60	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.80	nd	nd	nd	nd	nd	nd	nd	n
Wanstad Ditch	4/13/10	1400	8.84	nd	nd	3.37	36.6	nd	nd	nd	nd	nd	nd	nd	nd	176	28.8	nd	nd	nd	nd	nd	nd	1.9	n
	5/3/10	1530	nd	14.1	4.65	4.04	13.3	nd	nd	nd	nd	nd	13.5	nd	66.9	nd	259	17.0	51.3	nd	nd	13.9	nd	nd	n
	5/24/10	1345	nd	nd	nd	4.20	7.00	nd	5.00	nd	10.6	25.2	14.4	nd	46.2	nd	114	nd	154	nd	nd	15.6	nd	2.2	n
	6/16/10	1145	nd	nd	15.2	3.37	15.6	3.6	nd	35.8	nd	21.1	nd	nd	nd	nd	113	nd	47.4	nd	nd	nd	nd	5.5	n
	7/6/10	1340	nd	nd	nd	9.23	20.9	nd	nd	nd	nd	nd	5.49	nd	nd	nd	566	nd	31.9	nd	nd	nd	nd	40.9	n
	7/26/10	950	nd	nd	nd	31.0	11.6	nd	nd	nd	nd	nd	5.71	nd	nd	nd	84.1	nd	nd	nd	nd	nd	nd	3.9	n
	8/16/10	1115	nd	nd	nd	40.4	21.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	321	nd	nd	nd	nd	nd	nd	5.2	n
	9/9/10	1135	nd	nd	nd	3.43	11.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	71.3	nd	nd	nd	nd	nd	nd	nd	n
	9/29/10	907	nd	nd	5.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	40.4	nd	nd	nd	nd	nd	nd	nd	n
	10/18/10	1130	nd	nd	nd	3.0	4.60	nd	nd	nd	nd	nd	nd	nd	nd	nd	36.4	nd	nd	nd	nd	nd	nd	nd	68
	11/8/10	1050	nd	nd	2.60	3.60	9.40	nd	nd	nd	nd	nd	nd	nd	nd	nd	37.0	nd	nd	nd	nd	nd	nd	nd	n
	11/29/10	950	nd	nd	3.40	2.2	5.20	nd	nd	nd	nd	nd	nd	nd	nd	nd	20.4	nd	nd	nd	nd	nd	nd	nd	n

nd: not detected

Table S3. Composite pesticide concentration (µg/g wet weight) in sampled black fly larvae collected from reference sites (Cottonwood Creek, Dry Creek) and agricultural sites (Sand Run Gulch, Wanstad Ditch).

Site	Date	Larval composite mass (g)	Atrazine	Azoxystrobin	Boscalid	Ethalfluralin	Hexazinone	Imazalil	Metolachlor	Pendimethalin	Pyraclostrobin	Simazine	Trifluralin
Cottonwood Creek	2010-05-13	1.913	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.043	0.032
	2010-06-22	2.446	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.016	0.005
	2010-07-13	1.300	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.031
	2010-08-23	2.436	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.010	nd
	2010-09-15	0.698	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dry Creek	2010-06-21	0.541	0.180	nd	nd	nd	nd	nd	nd	nd	nd	0.234	nd
	2010-08-02	1.164	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.026	0.017
	2010-08-23	1.312	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.016
	2010-09-15	0.211	0.01	nd	nd	nd	nd	nd	nd	nd	nd	0.008	0.019
Sand Run Gulch	2010-05-03	1.192	nd	0.056	0.926	nd	0.083	0.181	nd	0.046	nd	nd	0.001
	2010-07-06	0.527	0.250	0.121	0.033	nd	0.096	0.326	0.441	0.018	nd	0.023	nd
	2010-07-26	0.730	nd	0.377	0.112	0.035	0.060	0.374	nd	0.252	0.43	0.062	0.043
	2010-09-09	0.226	0.064	0.428	0.090	0.009	nd	0.178	0.162	0.123	0.84	nd	0.013
	2010-09-29	1.499	0.097	0.136	0.106	0.016	nd	0.021	0.163	0.138	0.29	0.054	0.020
	2010-10-18	0.118	0.058	0.035	0.029	0.073	nd	0.066	0.106	0.088	0.16	0.078	nd
	2010-11-08	0.150	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Wastad Ditch	2010-08-16	0.485	nd	0.360	0.876	nd	0.100	0.277	0.294	0.009	0.190	nd	0.001

nd: not detected

Table S4. Estimated pesticide concentrations (µg/g wet weight) of individual black fly larvae collected from reference sites (Cottonwood Creek, Dry Creek) and agricultural sites (Sand Run Gulch, Wanstad Ditch).

Site	Date	Estimated individual larval mass (mg)	Estimated number of lavae per composite	Atrazine	Azoxystrobin	Boscalid	Ethalfluralin	Hexazinone	Imazalil	Metolachlor	Pendimethalin	Pyraclostrobin	Simazine	Trifluralin
Cottonwood Creek	2010-05-13	2.4	804	nd	nd	nd	nd	nd	nd	nd	nd	nd	34.4	25.8
	2010-06-22	2.6	954	nd	nd	nd	nd	nd	nd	nd	nd	nd	15.3	4.8
	2010-07-13	2.1	611	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	18.8
	2010-08-23	2.8	877	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.0	nd
	2010-09-15	2.4	286	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dry Creek	2010-06-21	1.0	557	99.4	nd	nd	nd	nd	nd	nd	nd	nd	131	36.5
	2010-08-02	1.0	1141	nd	nd	nd	nd	nd	nd	nd	nd	nd	29.3	19.5
	2010-08-23	0.9	1509	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	24.3
	2010-09-15	1.0	203	1.1	nd	nd	nd	nd	nd	nd	nd	nd	1.6	3.9
Sand Run Gulch	2010-05-03	1.6	751	nd	42.0	69.6	0.94	62.0	136	nd	34.5	nd	nd	0.85
	2010-07-06	1.4	369	90.9	44.8	12.3	0.14	35.3	120	163	6.8	nd	8.6	nd
	2010-07-26	1.4	504	nd	190	56.2	17.5	30.1	188	nd	127	215	31.2	21.7
	2010-09-09	1.6	161	9.1	60.8	12.8	1.3	nd	25.3	23.0	17.5	120	nd	1.8
	2010-09-29	1.6	944	86.6	129	100	14.9	nd	19.8	154	131	270	51.3	19.3
	2010-10-18	1.7	71	4.1	2.5	2.0	5.1	nd	4.7	7.5	6.2	11.5	5.5	6.7
	2010-11-08	1.9	80	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Wanstad Ditch	2010-08-16	0.8	616	nd	222	53.9	0.2	61.4	171	181	5.5	117	nd	0.53

nd: not detected

Table S5. Identified black fly larvae from 2010 samples. N refers to the number of black fly larvae identified from that site over the eight month sampling period (421 total).

Site	Species	Frequency	N
Cottonwood Creek	*Simulium piperi*	0.99	170
	Simulium canadense	0.01	
Dry Creek	*Simulium tuberosum*	0.07	135
	Simulium piperi	0.27	
	Simulium canadense	0.65	
Sand Run Gulch	*Simulium* sp.	0.88	101
	Simulium vittatum	0.12	
Wanstad Ditch	*Simulium* sp.	0.13	15
	Simulium vittatum	0.87	

Table S6. Mixed model results for thalli/μm^2 and spores/μm^2. The log of thalli and spores, (plus 5E-07) were modeled, with fixed effect of date, site, and date by site interaction. Due to insufficient numbers of black flies, Wanstad Ditch was not included in the model. Separate residuals were modeled for each date by site combination to accommodate unequal variation.

	Thalli/µm^2			**Spores/µm^2**		
Fixed Effects	Num,Den DF	F-Value	p-value	Num,Den DF	F-Value	p-value
site	1,121	4.2	0.043	1,218	0.01	0.905
date	16,121	170.16	<.0001	16,126	26.25	<.0001
site*date	9,96.1	31.97	<.0001	8,100	3.47	0.001

LIST OF FIGURES

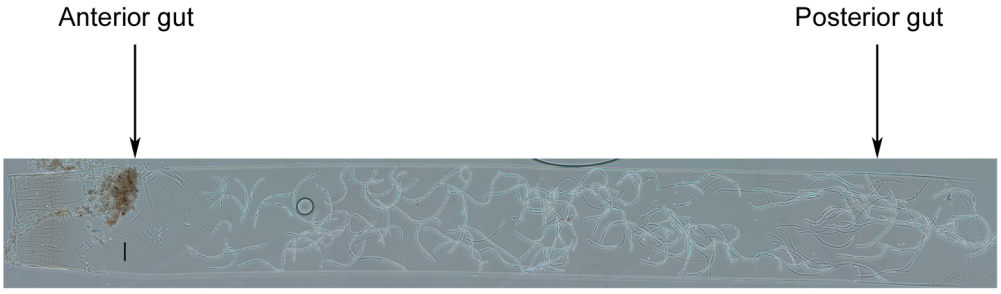

Figure S1. Composite image of black fly peritrophic matrix (PM) colonized by *Harpella* spp. from Cottonwood Creek (reference site) slide ID-84-E1. Scale bar = 100 μm.