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Authors
Pennington, Marcus
Rothman, Jason A
Jones, Michael
et al.

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Effects of contaminants of emerging concern on *Myzus persicae* (Sulzer, Hemiptera: Aphididae) biology and on their host plant, *Capsicum annuum*

Marcus John Pennington · Jason A. Rothman · Michael Bellinger Jones · Quinn S. McFrederick · Jay Gan · John T. Trumble

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Abstract Many countries are utilizing reclaimed wastewater for agriculture as water demands due to drought, rising temperatures, and expanding human populations. Unfortunately, wastewater often contains biologically active, pseudopersistant pharmaceuticals, even after treatment. Runoff from agriculture and effluent from wastewater treatment plants also contribute high concentrations of pharmaceuticals to the environment. This study assessed the effects of common pharmaceuticals on an agricultural pest, the aphid *Myzus persicae* (Sulzer, Hemiptera: Aphididae). Second instar nymphs were transferred to bell peppers (*Capsicum annuum*) that were grown hydroponically. Treatment plants were spiked with contaminants of emerging concern (CECs) at environmentally relevant concentrations found in reclaimed wastewater. *M. persicae* displayed no differences in population growth or microbial community differences due to chemical treatments. Plants, however, displayed significant growth reduction in antibiotic and mixture treatments, specifically in wet root masses. Antibiotic treatment masses were significantly reduced in the total and root wet masses. Mixture treatments displayed an overall reduction in plant root wet mass. Our results suggest that the use of reclaimed wastewater for crop irrigation would not affect aphid populations, but could hinder or delay crop production.

Keywords CECs · Microbial communities · Pollution · Antibiotics · Wastewater

M. J. Pennington · J. Gan · J. T. Trumble
Graduate Program in Environmental Toxicology, University of California, Riverside, CA 92521, USA

M. J. Pennington (✉) · J. A. Rothman · M. B. Jones · Q. S. McFrederick · J. T. Trumble
Department of Entomology, University of California, 417 Entomology Building, Riverside, CA 92521, USA
e-mail: mpenn001@ucr.edu

J. A. Rothman · Q. S. McFrederick
Graduate Program in Microbiology, University of California, Riverside, CA 92521, USA

J. Gan
Department of Environmental Science, University of California, Riverside, CA 92521, USA

Introduction

According to the National Center for Health Statistics (2014), there have been increasing pharmaceutical prescriptions for the past 30 years, and they have almost tripled in the past 14 years alone (Schumock et al. 2014). For agriculture, in 2013 over 6.6 million kg of the 9.1 million kg of antibiotics used were to increase production (Department of Health and Human Services 2013). Common "contaminants of emerging concern" (CECs) (pharmaceuticals, antibiotics, mental stimulants, and surfactants) can be excreted by both humans and animals with little or no change in the chemical structures (Hirsch et al. 1999; Ternes et al. 2004; Yamamoto et al. 2009) and, not surprisingly, they have been appearing in wastewater, and in some cases tap water, over the past few years (Monteiro and Boxall 2013; Sklerov and Saucier 2011) (Table 1).

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Table 1 Contaminants of emerging concern (CEC) treatment group components and concentration

Contaminant	Concentration (μg/L)	Reference
Antibiotics		
Oxytetracycline	72.90	(Kolpin et al. 2002)
Lincomycin	0.730	(Kolpin et al. 2002)
Ciprofloxacin	6.500	(Mutiyar and Mittal 2014)
Hormones		
17α--Ethynylestradiol	0.831	(Kolpin et al. 2002)
17β-Estradiol	0.200	(Kolpin et al. 2002)
19-Norethindrone	0.872	(Kolpin et al. 2002)
Estrone	0.112	(Kolpin et al. 2002)
Mixture		
Acetaminophen	10.00	(Kolpin et al. 2002)
Caffeine	6.000	(Kolpin et al. 2002)
Antibiotics	Concentration as above	
Hormones	Concentration as above	

Most wastewater treatment facilities are not capable of removing all pharmaceuticals (Gros et al. 2010; Hedgespeth et al. 2012), resulting in these compounds being found in effluent. In addition, during heavy storms, untreated wastewater overflow can release even higher concentrations of some pharmaceuticals, which then directly contaminate the environment (Phillips et al. 2012). Many of these compounds can be found at biologically active concentrations in surface waters around the world (Alvarez et al. 2013; Fine et al. 2003; Huang et al. 2013; Kolpin et al. 2002; Mutiyar and Mittal 2014; Shappell et al. 2007; Wei et al. 2011). In addition, there is also an increased effort to use reclaimed wastewater in drought-affected areas (Brown et al. 2013; Wu et al. 2012), resulting in increased exposure. In agriculture/livestock operations, pharmaceuticals are found in manure that is used as fertilizer for feed and crops, effectively compounding the pharmaceutical concentrations (Kumar et al. 2005; Shappell et al. 2007; Wei et al. 2011). Current research shows that these chemicals tend to be both pseudopersistant in soil and detrimental to soil microbes (Alvarez et al. 2013; Chefetz et al. 2008; Gan et al. 2012; Kinney et al. 2006; Thiele-Bruhn 2003).

Our recent studies of the effects of pharmaceuticals on aquatic insects show that at concentrations found in reclaimed water, CECs can alter development of the medically important mosquito *Culex quinquefasciatus*, its susceptibility to a common larvicide, and its larval microbial communities (Pennington et al. 2015, 2016). Female *Megaselia scalaris*, which are ecologically important detritivores, also displayed an increased developmental period, which could jeopardize the population's survival when exposed to CECs (Pennington et al., 2017a, b). Also, the common agricultural pest *Trichoplusia ni* (cabbage looper) was negatively affected by antibiotics through a plant matrix (Pennington, Rothman, Dudley, et al. 2017). A common birth control agent, 17α-ethinylestradiol, and Bisphenol-A, a common plasticizer, have been shown to cause deformities in the midge *Chironomus riparius* (Watts et al. 2003). However, aquatic insects' constant exposure to these CECs is likely greater than most terrestrial insects. Interestingly, many CECs were not designed to specifically to impact microbes but have been shown to affect microbial communities. For example, the mental stimulant caffeine can alter biofilm respiration, and diphenhydramine, an antihistamine, has been shown to modify the microbial community of lake biofilms (Rosi-Marshall et al. 2013). Therefore, accurately predicting the consequences of specific CECs, even in model insects, currently is difficult if not impossible. This problem is exacerbated by a general lack of information regarding effects of pharmaceuticals and other CECs on the microbial communities of terrestrial insects.

Arthropods, such as insects and crustaceans, rely on hormones to grow, develop, mate, and produce pigmentation (Jindra et al. 2013; Knowles and Carlisle 1956; Martín et al. 2001). However, many pharmaceuticals, especially mammalian sex hormones, are structurally similar to chemicals that these organisms rely on for development. These pharmaceuticals bind to receptors and either increase or disable their counterparts' natural function. Endocrine disruption has been noted in birds, reptiles, and arthropods, primarily in the modification of primary and secondary sexual characteristics, and changes in courtship behaviors (Gonzalez et al. 2001; Hoffmann and Kloas 2012; Jindra et al. 2013; Segner et al. 2003; Tompsett et al. 2012, 2013). While most arthropod hormones do not closely match those of mammals, their molting hormone (ecdysone) is very

similar in structure to the mammalian female sex hormone 17β-estradiol. Increased molting events and inhibition of chitobiase, the enzyme responsible for digestion of the cuticle during insect molting, have been noted in crustaceans exposed to mammalian hormones (Rodríguez et al. 2007; Zou and Fingerman 1997). In addition to these effects, pharmaceuticals have been shown to have delayed cross-generational effects (Watts et al. 2001).

Aphids are phloem-limited hemimetabolous insects (immature insects resemble the adults and do not undergo complete metamorphosis). *Myzus persicae* (Sulzer, Hemiptera: Aphididae) is polyphagous, highly cosmopolitan, and an efficient vector of plant viruses (van Emden and Harrington 2007). This insect overwinters in the egg stage on *Prunus* species, and when their host plants are over-populated and/or stressed, they begin producing alates (winged forms) to disperse and colonize new plants (Davis and Landis 1948; Ponsen 1977; Sorensen 2009; Sylvester 1954; Taylor 1908). The sexual forms are also alates and are formed in autumn temperatures wherever peaches or suitable host plants are available (Sorensen 2009; van Emden and Harrington 2007). Economically, *M. persicae* is most damaging in the spring, when the insects hatch and feed on new peach leaves, and serve as vectors of over 100 different plant viruses (both persistent and non-persistent) (van Emden and Harrington 2007). The aphid microbiome has been extensively studied and is well understood, making aphids excellent models for microbial community and biological research (Davis and Landis 1948; Singh and Singh 2016; Sorensen 2009; Sylvester 1954). Previous research has determined that antibiotics can reduce fecundity, reduce population growth, and increase mortality of aphids (Baumann et al. 2013; Douglas 1998; Harries and Mattson 1963; Jayaraj et al. 1967). Previous findings were usually due to the reduction of *Buchnera*, a key symbiont that provides required nutrients the aphids cannot make themselves or acquire from their diet (Douglas 1998).

Currently, there is no information regarding pharmaceutical effects at the concentrations found in reclaimed water on the growth and development of phloem-limited insects or their microbial community composition. Many herbivores can be exposed to these contaminants after the CECs enter surface waters, soil, and plants from wastewater reuse and unintended discharge. There is minimal information available regarding effects of CECs when translocated through plants to terrestrial insects, especially those with specialized feeding techniques. Depending on the acquisition and sequestration by their host-plant species, insects with phloem-limited feeding methods, such as aphids, could have either reduced or increased exposure to CECs. Because previous research demonstrated a substantial change in both the biology and microbial communities of other insects when treated with ecologically relevant levels of CECs (Pennington et al. 2015, 2016; Pennington, Rothman, Dudley, et al. 2017; Pennington, Rothman, Jones, et al. 2017), and since aphid growth and development rely on symbionts, we hypothesized that aphids could be affected in similar ways. To test this hypothesis, we conducted bioassays of aphids reared on a key host plant, *Capsicum annuum*, exposed to CECs at concentrations found in reclaimed water. Any effects would have potentially important implications from agricultural perspectives. Also, as there is currently little information on effects of CECs on terrestrial insects acquired through a plant matrix, our findings would have possible interest for integrated pest management (IPM) research.

Methods and materials

Insect rearing

Green peach aphids (*M. persicae*) were obtained from a colony maintained on bell peppers (*C. annuum*, variety "Islander") in a University of California Riverside greenhouse at 25 ± 2 °C. The insects were in colony for less than 1 year at the time of the experiments. Natural light was supplemented with artificial light to maintain a long-day photoperiod (LD 16–8). When transfer of insects was required, second instar aphids were moved to new host plants to eliminate mortality that occurred when first instar insects were handled.

Population growth

Bell peppers were grown from seeds in 10.16-cm^2 pots in UC soil mix no. 3 (Matkin and Chandler 1957) and fertilized with Miracle Gro nutrient solution (Scotts Company, Marysville, OH) at labeled rate and watered as needed in the UCR greenhouse. When plants were approximately 10 cm tall, their roots were washed with D.I. water and they were transplanted to 475-mL Mason jars (Fischers, IN). Mason jars were coated with Folk

Art Multi-Surface acrylic paint (Plaid Enterprises, Inc., Norcross, GA) on the outside to prevent root exposure to light. Jars were filled with hydroponic growth media (Oasis Hydroponic Fertilizer 16-4-17, Oasis Grower Solutions, Kent, OH) containing CEC concentrations described in Table 5.1 with average pH of 7.0 ± 0.5 as in Pennington, Rothman, Dudley, et al. (2017).

Treatment media were prepared utilizing stock solutions of treatment compounds dissolved in 5:45 (*v*:*v*) methanol:D.I. water with aliquots of < 500 μL being dissolved in 18 L. Growth media were stored at room temperature in blackened 19-L tanks to protect the CECs from photodegradation and to prevent algal growth. Hydroponic growth media were drained, by Erlenmeyer filter flask and vacuum, and replaced every 3 days to hinder bacterial and fungal growth and maintain CEC concentrations. After filtering through a HEPA-CAP (Whatman, Inc., Florham Park, NJ) air filter, house air was bubbled into jars through black irrigation tubing to aerate the hydroponic growth media. Each container included one of five CEC treatments or an untreated control hydroponic solution, and was used to water four plants. Plants grew 3 weeks before 10 *M. persicae* were placed evenly on two fully expanded leaves per plant. There were four replicate hydroponic containers for each of the six treatments ($n = 20$ individuals per plant; $n = 480$ total *M. persicae*). Data regarding population growth were collected daily and the experiment was ended after 2 weeks. Three life-stage groupings (first and second, third and fourth, and adult life stages) were collected from each plant, with a minimum sample size of 20 individuals per life stage ($n = 20$ individuals per plant; $n = 480$ total *M. persicae*), and stored in 200 proof ethanol at 62 ± 2 °C until DNA extractions were performed. Plants were separated into parts (roots and leaves), weighed, and immediately frozen at − 62 ± 2 °C.

DNA extractions and Illumina sequencing of whole body *M. persicae* bacteria

All DNA extractions and Illumina preparations were performed as in McFrederick and Rehan (2016) within 1 month of − 62 ± 2 °C storage. Briefly, DNA extractions were performed using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Five pooled individuals from each life stage ($n = 3$), each treatment group ($n = 6$), and replicate group ($n = 4$), along with triplicates of a pooled blank for each treatment group ($n = 9$) and three negative blanks ($n = 3$; total $n = 84$), were placed in individual wells of a 96-well plate provided in the kit and extracted per the kit's directions.

Dual-index barcoding was used to prepare libraries for sequencing on the MiSeq sequencer (Illumina Inc., San Diego, CA). Primers that included either the Illumina sequencing primer, a unique eight-nucleotide long barcode, and the forward or reverse genomic oligonucleotide were used as in Kembel et al. (2014), and the bacterial 16S rDNA sequence primers used were 799F-mod3 CMGGATTAGATACCCKGG (Hanshew et al. 2013) and 1115R AGGGTTGCGCTCGTTG (Kembel et al. 2014). One microliter of clean PCR product was used as a template for the next PCR, using the primers PCR2F (CAAGCAGAAGACGGCATAC GAGATCGGTCTCGGCATTCCTGC) and PCR2R (AATGATACG GCGACCACCGAGATCTACAC TCTTTCCCTACACGACG) as in Kembel et al. (2014). The PCR products were then normalized using SequalPrep Normalization plates (ThermoFisher Scientific, Waltham, MA). Five microliters of each normalized sample was pooled together, and a 2100 Bioanalyzer (Agilent, Santa Clara, CA) was used to assess library quality. Libraries were then sequenced using a MiSeq sequencer with 2X 300 cycles. Raw data are available on the NCBI Sequence Read Archive (SRA) accession number SRR5929442.

Bioinformatics

All genomic data were processed in macQIIME version 1.9.1-20150604 (Caporaso et al. 2010; Kuczynski et al. 2012) as in Pennington, Rothman, Dudley, et al. (2017). The R package "gplots" (Warnes et al. 2016) was used to create heatmaps of the most abundant bacterial families; a top ten abundance was used as the cutoff.

Statistics

All statistical analyses were performed using R. Normality was determined using Shapiro-Wilk normality tests, quantile-quantile plots, and histograms. Effects of treatments on population growth were determined using a generalized linear model, and post hoc tests were performed using R's "summary" function. In all cases when data were not considered normal, either a Poisson distribution or a negative binomial generalized linear model was used and best fit was determined from Akaike's "An Information Criterion" and followed with

R's summary function for pairwise comparisons of treatment. Adonis within the R package "vegan" (Oksanen et al. 2008) was used for all PERMANOVA analyses. All Adonis analyses were conducted on weighted UniFrac distance matrices.

Results

Aphids reared on treated pepper plants showed no difference in population growth (Fig. 1) (χ^2 4.68; *df* 5; p = 0.46). There was significant reduction of the total mass of the peppers (χ^2 12.94; *df*: 5; $p = 0.024$) specifically in the antibiotic (t value = − 2.18; $p = 0.043$) treatments (Fig. 2). When dissected into parts, there were significant differences in leaf (χ^2 12.90; *df*: 5; $p = 0.024$) and root mass (χ^2: 13.52; *df*: 5; $p = 0.019$; Figs. 2 and 3). For root masses, differences were predominantly in the antibiotic (t value = − 2.81; $p = 0.012$) and mixture (t value = − 2.32; $p = 0.033$) treatments (Fig. 4).

The most dominant family in the aphid microbial community was *Enterobacteriaceae* (genus *Buchnera*; Fig. 5) across all treatments (accounting for at least 84%; Table 2) and all life stages (accounting for at least 82%; Table 3).

Discussion

Our work demonstrates that the selected CECs did not affect population dynamics or microbial communities of *M. persicae* reared on bell peppers. Many plants will translocate CECS (Wright et al. 2012; Wu et al. 2012, 2014, 2015). However, some plants can metabolize and/

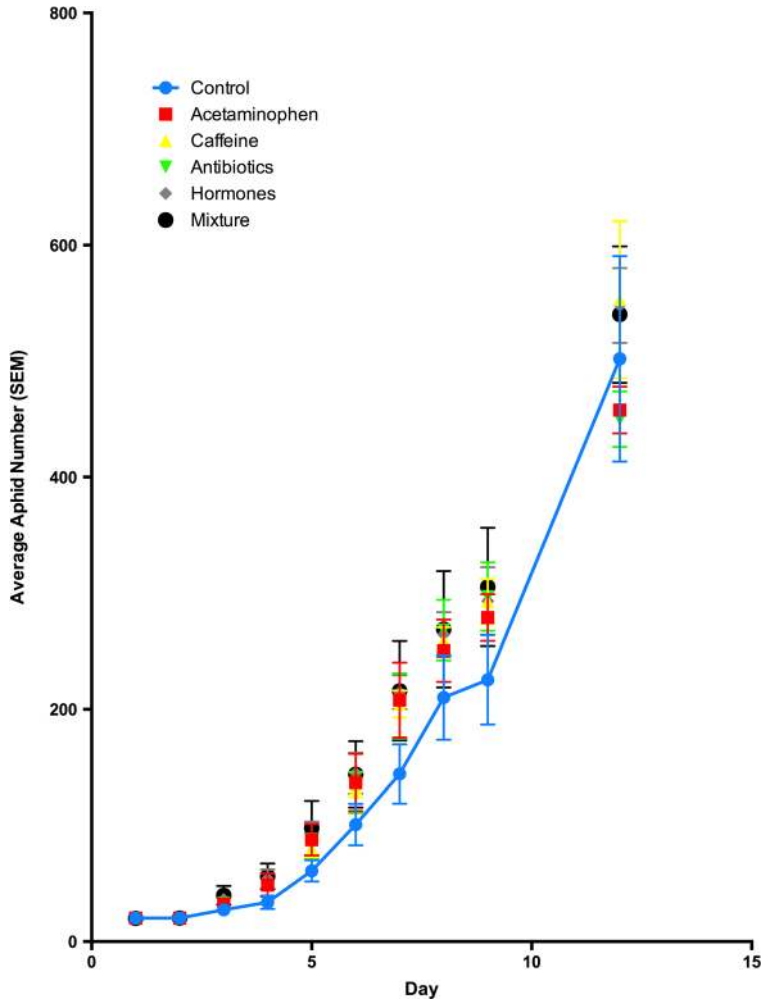

Fig. 1 Average *Myzus persicae* population in each treatment by day. *** denotes a significant (α < 0.05) difference relative to the control

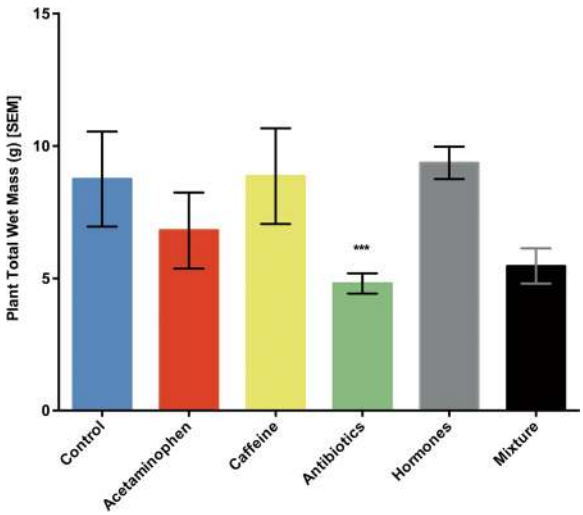

Fig. 2 Average wet mass of whole plants by treatment. *** denotes a significant ($\alpha < 0.05$) difference relative to the control

or sequester xenobiotics in tissues other than phloem, thereby removing the CEC exposure to aphids (Huber et al. 2009; Wu et al. 2015). As aphid species rely heavily on the endosymbiont *Buchnera* species to grow and develop, many aphid populations treated with high concentrations of antibiotics will not survive (Harries and Mattson 1963; Jayaraj et al. 1967). However, aphid microbial communities were not affected when treated with antibiotics and other CECs at the low concentrations found in reclaimed water, which is possibly why

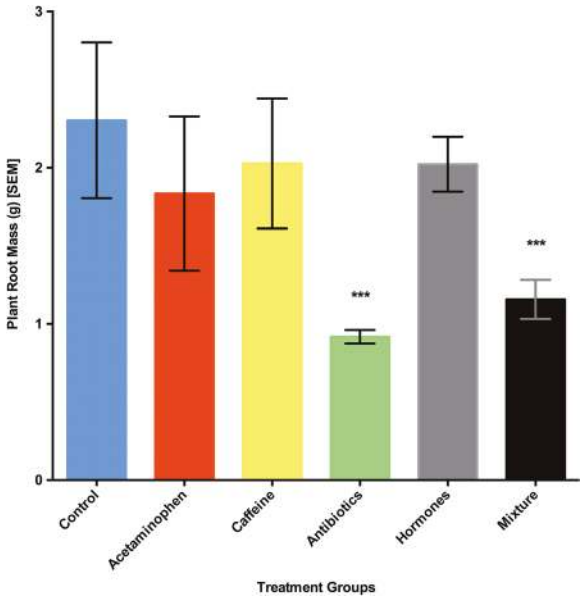

Fig. 3 Average root wet mass. *** denotes a significant ($\alpha < 0.05$) difference relative to the control

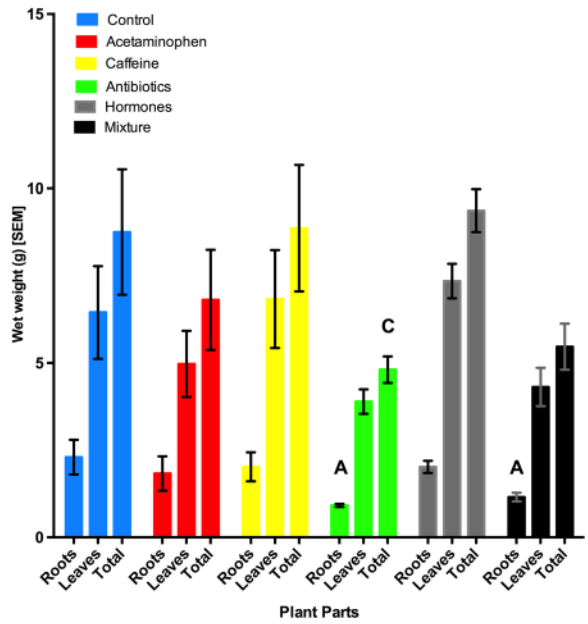

Fig. 4 Average wet masses of plants as total and plant parts by treatment. Letters denote a significant ($\alpha < 0.05$) difference between the column and the relative control

there were no discernable effects on the aphid population as a whole. While treatments used in our study have previously been demonstrated to have negative effects for at least two other species of insects (Pennington et al. 2015, 2016; Pennington, Rothman, Jones, et al. 2017), this work suggests that aphids are either not exposed to CECs through their host plant, or their bacterial symbionts are not sensitive to them, or depleted enough, to alter their basic biology.

Plants treated with antibiotics typically have lower levels of intracellular calcium due to chelation (Bowman et al. 2011). However, in our study, we did not notice any obvious signs of calcium stress (discoloring or death of leaves), possibly due to the use of a hydroponic solution which contains more than enough metal ions to provide adequate nutrients to the plants, even with some chelating. We did notice an overall decrease in mass for plants treated with antibiotics likely due to a slowed growth rate from direct action of the antibiotics on plant growth (Yu et al. 2001).

Overall, there were no discernible effects of CECs on aphid growth and survival or the key bacteria in their microbial community. However, there were reductions in plant growth when even relatively low concentrations of antibiotics were included in their water. This could pose a problem for growers because antibiotics tend to be reapplied with each watering and if manure from

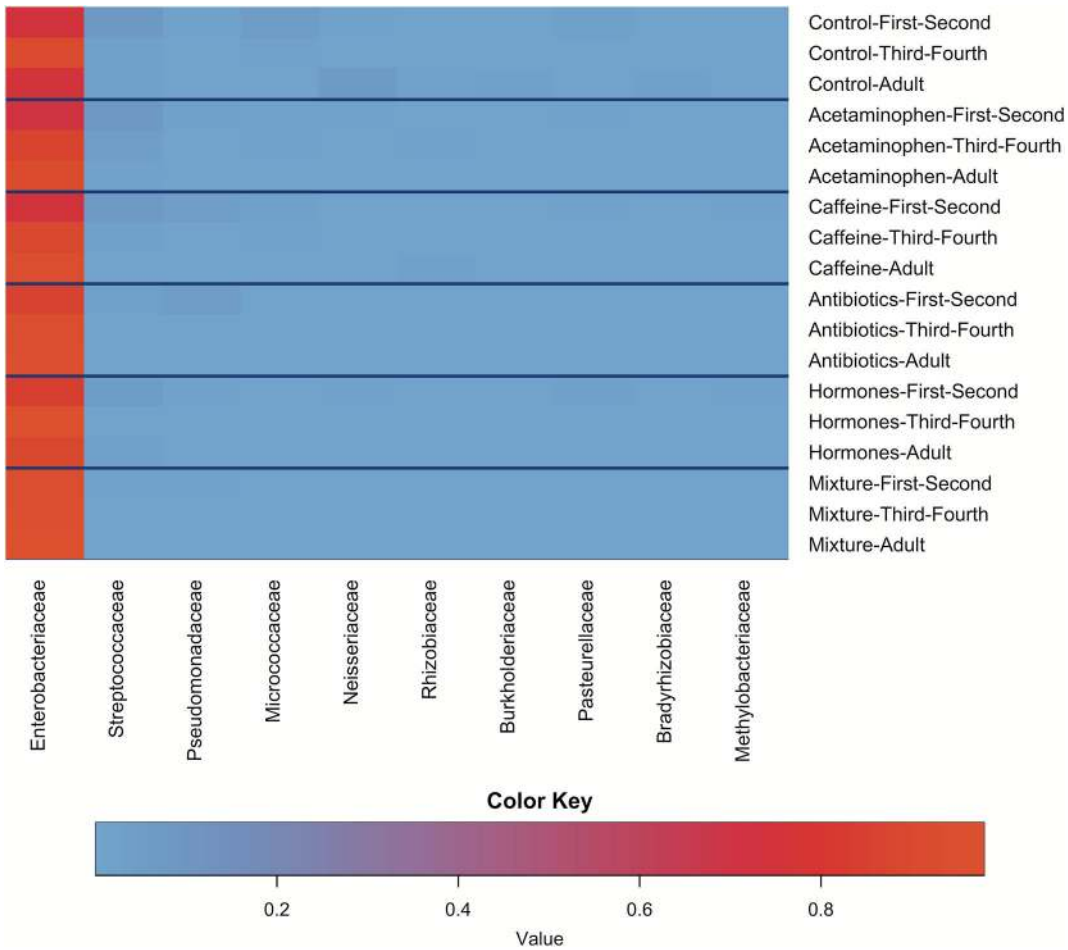

Fig. 5 Heatmap of the top 10 most proportionally abundant bacterial families by average OTUs of treatment life-stage pairing. Increased red coloration is indicative of increased proportional abundance

Table 2 Percentages of the top 10 bacterial families in each treatment (incorporating all life stages)

Family	Control	Acetaminophen	Caffeine	Antibiotics	Hormones	Mixture
Enterobacteriaceae	80.52	84.69	86.48	91.83	91.08	95.31
Streptococcaceae	4.35	3.72	3.07	0.96	2.17	0.64
Pseudomonadaceae	1.01	1.10	1.36	2.03	0.58	0.91
Micrococcaceae	2.43	0.75	1.00	0.10	0.40	0.19
Neisseriaceae	2.70	0.76	0.29	0.07	0.59	0.05
Rhizobiaceae	0.38	0.78	1.38	0.27	0.20	0.08
Burkholderiaceae	1.12	0.63	0.54	0.21	0.37	0.20
Comamonadaceae	0.40	0.47	0.75	0.41	0.46	0.30
Pasteurellaceae	0.98	0.71	0.43	0.06	0.71	0.03
Bradyrhizobiaceae	0.79	0.65	0.45	0.42	0.26	0.24
Total percentages	94.69	94.26	95.75	96.36	96.82	97.95
Total reads	77,031	95,718	117,524	104,246	117,125	93,503

Table 3 Percentages of the top 10 bacterial families in each life stage (incorporating all treatments)

Family	First-second	Third-fourth	Adult
Enterobacteriaceae	82.21	93.14	91.31
Streptococcaceae	4.62	1.34	1.09
Pseudomonadaceae	2.49	0.49	0.39
Micrococcaceae	1.53	0.44	0.19
Neisseriaceae	0.90	0.17	0.83
Rhizobiaceae	0.25	0.46	0.91
Burkholderiaceae	0.44	0.24	0.75
Comamonadaceae	0.79	0.39	0.23
Pasteurellaceae	1.02	0.22	0.12
Bradyrhizobiaceae	0.20	0.33	0.82
Total percentages	94.45	97.22	96.63
Total reads	216,040	182,754	206,353

antibiotic-treated animals is used as fertilizer (Chari and Halden 2013; Wu et al. 2010; Wu et al. 2015). These antibiotics can reportedly hinder the growth of the plant's rhizosphere which would create additional problems when crops are rotated to reintroduce nitrogen into the soil (Kong et al. 2006). More studies will need to be performed to determine how CECs will affect root microbial communities in soil, the roots themselves in soil, and degradation of CECs in soil. Nonetheless, the results are immediately applicable to hydroponic cropping systems.

Conclusion

Results from this study add new information to the limited literature reporting effects on plants and various organisms. Plants (*C. annuum*) were negatively affected by pharmaceuticals present in their hydroponic media. Plant wet masses (both root and total mass) were reduced by the pharmaceuticals, specifically in the antibiotic and the mixture treatments. However, the treatments had no discernable effects on the aphid *M. persicae* reared on these treated plants. This is surprising, as much of the literature to date has found negative effects on insects treated with these pharmaceuticals. We propose that the plant matrix, potentially acting as a dilution factor, along with the specialized phloem-feeding strategy of the insect, provides some measure of protection through a reduction in exposure. More studies will need to be conducted to discern the concentration of CECs in the phloem and the apparent resistance of some genera of bacterial symbionts like *Buchnera* to these pollutants.

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