Abstract: Pollen shedding can produce rapid, abundant exchanges of nutrient-rich biomass from plant canopies to the surface. When pollen deposits onto understory plants, it can be washed off during storms via throughfall (a drip flux) and stemflow (a flux down plant stems). Pollen deposition may also alter the organismal community on plant surfaces, changing other biological particulates transported by throughfall and stemflow. We report concentrations and fluxes of pollen and other biological particulates (flagellate cells, nematodes, rotifers, mites and hexapodans) in throughfall and stemflow from an understory forb, *Eupatorium capillifolium* (Lam. dogfennel), during a *Pinus palustris* (Mill. longleaf pine) pollen shedding event, then compare these results to observations collected when pollen was absent. Pollen flux was 95.6 x 10^6 grains ha\(^{-1}\) season\(^{-1}\) from dogfennel canopies (63% and 37% transported by throughfall and stemflow, respectively), representing 0.1-3.2 g ha\(^{-1}\). Median concentrations in flagellates, nematodes and rotifers for throughfall and stemflow were higher during pollen shedding; however, mites and hexapodan concentrations were similar regardless of pollen presence. This is the first report of flagellate and hexapodan concentrations in canopy drainage waters. Flagellate concentrations were higher than for other organisms—being similar to those reported for streams, 10^5-10^7 cells L\(^{-1}\)—and hexapodan fluxes were ~50 individuals m\(^{-2}\) per 1 cm of rainfall. These results indicate that throughfall and stemflow can (i) transport ecologically relevant amounts of pollen and organisms from the phyllosphere to the surface, and (ii) that the composition and flux of biological particulates can change markedly during pollen shedding.

Key words. Pollen, Phyllosphere, Rainfall, Precipitation partitioning, Metazoans, Particulates.
Introduction.

Forest canopies can shed $10^1$-$10^3$ kg ha$^{-1}$ of pollen over a short period of time—days, weeks or months—depending on the plant species and meteorological conditions (e.g., Boyer, 1981; Doskey and Ugoagwu, 1989; Greenfield, 1996; Lee et al., 1996; Cho et al., 2003; Lee and Booth, 2003). This relatively rapid and abundant canopy-to-surface exchange of biomass can play important roles in the biogeochemistry of the canopy and receiving aquatic and terrestrial systems. Pollen macronutrient concentration is relatively high, being 2-5 times greater than litterfall (Stark, 1973; Lee et al., 1996). The unique stoichiometry of pollen can result in the release of biolabile material after deposition to detrital and freshwater systems (Rösel et al., 2012; Masclaux et al., 2013; Filipiak, 2016). Pollen is enriched in N and (especially) P compared to other terrestrial materials. In fact, pollen deposition has been estimated to recycle ~20 kg-N ha$^{-1}$ y$^{-1}$ (Perez-Moreno and Read, 2001)—an amount that exceeds annual N recycled by litterfall in some forests (Greenfield, 1999)—and the few observations to date suggest pollen is readily mineralized (Greenfield, 1999; Webster et al., 2008). Pollen P concentrations can be three times those of desert dust aerosols (Bigio and Angert 2018) and pollen P also may be rapidly released by microbial communities (Graham et al., 2006; Filipiak, 2016).

A portion of shed pollen will not directly access the litter layer or nearby aquatic systems, because many particles will deposit onto the canopy surfaces of nearby and understory vegetation (Millerón et al., 2012). This has nontrivial effects on the phyllosphere. Pollen can contain unique microbial communities (e.g., Ambika Manirajan et al., 2016; Kim et al., 2018) and, thus, the coating of the phyllosphere by pollen shedding events can influence the resident microbial community. Moreover, fungi colonizing the phyllosphere parasitize pollen
particles (e.g., Hutchison and Barron, 1997; Magyar et al., 2018). Pollen also ephemerally alters the invertebrate community visiting the phyllosphere, i.e., pollinators (Aleklett et al., 2014; Kwon et al., 2018). Thus, rainfall during and after pollen events is hypothetically altered in its particulate composition by draining through this transitorily unique phyllosphere—beyond the simple washing of pollen particles. To the authors’ knowledge, however, no research has tested this hypothesis to date.

The passage of rainwater through plant canopies generally results in a significant transfer of solutes and particulates (Ponette-Gonzalez et al., 2020), both as a drip flux from canopy surfaces (throughfall) and a contact flow down the outside of plant stems (stemflow). During (non-pollen) storms, throughfall and stemflow have been reported to transport to the forest floor quadrillions of bacterial cells ha\(^{-1}\) (Bittar et al., 2018), billions of fungal spores ha\(^{-1}\) (Van Stan et al., 2021), and stemflow alone has been estimated to transport millions of metazoans ha\(^{-1}\) (Ptatscheck et al., 2018). In this short communication, we present and briefly discuss pollen, flagellated protist, and invertebrate animal concentrations and fluxes in throughfall and stemflow from a common and North American understory and pasture forb, *Eupatorium capillifolium* Lam. (dogfennel) during a *Pinus pallustris* Mill. (longleaf pine) pollen shedding event (February-March 2019).

**Methods.**

The study was conducted in a forest fragment in Statesboro, Georgia, USA, at Georgia Southern University’s main campus (32.430 N, -81.784 W, 65 m A.S.L.). Climate is subtropical (Köppen Cfa), 30-year mean annual precipitation is 1,170 mm y\(^{-1}\) spread relatively evenly
throughout the year (University of Georgia, 2019). The overstory is dominated by *P. palustris* (223 trees ha\(^{-1}\)) and the understory is dominated by dogfennel (56,770 stems ha\(^{-1}\)). See Gordon et al. (2020) for more information on the study site. Pollen shedding from *P. palustris* occurred at the site during February-March 2019. During this time, 5 rain events occurred whereafter stemflow and throughfall water samples were collected from the dogfennels. For comparison, water samples were collected from 2 storms during non-pollen conditions (in October 2019).

Three dogfennel clumps were randomly selected for throughfall and stemflow monitoring. Within these three clumps, 30 individual dogfennel stems were randomly selected for stemflow monitoring. Throughfall gauges consisted of 9 randomly placed funnels (506.7 cm\(^2\) collection area each)—three per dogfennel clump (1,520.1 cm\(^2\) total collection area per clump)—connected to HDPE bottles that were manually measured with graduated cylinders immediately after a storm ended (within 4 h). Stemflow collars were constructed from aluminum foil, 15-mm inner-diameter flexible polyethylene tubing, electrical tape, and silicon thinned with hydrotreated light (95-100%) naphtha (VM&P Naphtha, Klean-Strip, Memphis TN USA) (same as Gordon et al., 2020). Stemflow volume was measured with a graduated pipette (with 1 mL graduations) from 500 mL plastic bottles connected to the tubing base. All samplers were pre-cleaned with pH 2 ultrapure water, triple-rinsed, air dried, and covered until the start of a rainfall event. All samples collected for pollen, flagellated protist, and invertebrate analysis were immediately placed into refrigeration (~4°C) until being processed. Three volume-weighted composite samples of stemflow and throughfall—one for each dogfennel clump—were examined per storm.
Average pollen density per water sample was determined by examining three or four (depending on total volume) 30 µl subsamples using a compound light microscope (Motic BA210E) at 100x total magnification. Similarly, the per sample average unicellular flagellate density was determined for three or four 10 µl subsamples counted using a hemocytometer viewed under the microscope at 400x total magnification. All water samples were mixed via a vortexer (FisherBrand Analog Vortex) for approximately 5 s prior to taking each subsample. Invertebrates were quantified by examining the entire remaining water sample in small, subsectioned portions using the dark field setting of a dissecting microscope (Olympus SZX16) and a minimum total magnification of 40x.

Data were analyzed for descriptive statistics of central tendency and variability. To assess whether and to what extent correlation exists between particulate types, Spearman ρ tests were performed between mean pollen, flagellate, and invertebrate densities L⁻¹ of throughfall and stemflow. No statistical difference testing was performed due to the limited number of samples examined per storm. All statistical analyses were done in JMP Pro v. 13.

**Results and discussion.**

During pollen shedding, throughfall and stemflow generally had concentrations of 1-6 x 10⁵ pollen grains L⁻¹, but zero-to-negligible pollen concentrations were observed in October (Figure 1a). Overstory throughfall estimations (from the *P. palustris* overstory) ranged from 3.5-44.1 mm event⁻¹ and dogfennel canopies partitioned this into 36-76% throughfall and 5-67% stemflow per event (Table S1, Supplemental Materials). Note that the 67% stemflow estimate resulted from additional occult precipitation. Given these water flux estimates, the total pollen
flux across the 5 storms was 95.6 x 10^6 grains ha^-1, where 63% and 37% were transported by throughfall and stemflow, respectively. To estimate pollen mass flux draining from the dogfennel understory, we could find no reports of individual pollen grain mass for *P. palustris*. However, an estimate of individual pollen grain mass may be computed from observations of density, 0.45-0.58 ng pL^-1 from several *Pinus* species (Durham, 1946; Hirose and Osada, 2016), and particle volume, 16.3-57.4 pL also for several *Pinus* species (Kim et al., 2018). These observations suggest a minimum and maximum weight of 7.3 and 33.4 ng grain^-1 for *Pinus* pollen, indicating that the pollen mass flux in net rainfall just from the dogfennel canopy may be 0.1-3.2 g ha^-1. This flux is modest compared to the kg ha^-1 of total pollen deposition from pine forests (Lee et al., 1996; Cho et al., 2003; Lee and Booth, 2003), but this is perhaps unsurprising as some portion certainly reaches its intended destination (cones), directly deposits to the surface or into waterbodies (e.g., Graham et al., 2006), or reaches the surface via other plants’ throughfall and stemflow. Still, we note that throughfall and stemflow fluxes are highly spatially variable and may locally concentrate pollen inputs to small areas at the surface (Van Stan et al., 2020). For dogfennel plants at this site, the coefficient of variation for throughfall and stemflow water fluxes were 38% and 254% for rainstorms, respectively—and higher for storms with occult precipitation (Gordon et al., 2020). Dogfennel throughfall was observed to exceed stand-scale overstory throughfall by 190%. The especially high spatial variability observed for stemflow resulted in median water inputs near individual plant stems 18-200 times more concentrated than the stand-scale overstory throughfall (Gordon et al., 2020). Given that pollen grains are rich in N and P (Stark, 1973; Lee et al., 1996; Cho et al., 2003) and appear to be bioavailable (Greenfield, 1999; Webster et al., 2008; Filipiak, 2016),
Figure 1. Descriptive statistics for concentrations of pollen and biological particulates in throughfall (TF) and stemflow (SF) when pollen was present and absent. Line is the median; Boxes are the interquartile range and lines mark the 10% and 90%.
spatial concentrations of pollen and water inputs by stemflow and throughfall drip points may be notable localized nutrient subsidies to soils.

Throughfall and stemflow were enriched with other biological particulates, some of which were observed to co-vary with pollen grain presence. There were marked reductions in the concentration of flagellate cells when pollen was absent, where median concentration decreased from $4.8 \times 10^7$ to $9.7 \times 10^6$ cells L$^{-1}$ for stemflow and $2.7 \times 10^6$ to 0 cells L$^{-1}$ for throughfall (Figure 1b). Median concentrations in nematodes and rotifers were also higher during pollen shedding, especially for stemflow where the median concentration decreased by 67% and 76% in the absence of pollen for nematodes and rotifers, respectively (Figure 1c-d).

Results suggest that pollen deposition may alter the canopy environment such that it supports a larger community of these organisms. Alternatively, since dogfennels begin to senesce leaves in the fall, observed changes in these organismal fluxes in stemflow may relate to seasonal shifts in canopy morphology. Mite and hexapodan (insect and collembolan) concentrations were relatively similar for throughfall (means being 3 mites L$^{-1}$ and 11 hexapodans L$^{-1}$) and stemflow (means being 4 mites L$^{-1}$ and 11-13 hexapodans L$^{-1}$) regardless of pollen presence (Figures 1e-f). Spearman $\rho$ correlations were moderately strong between pollen and both flagellates and nematodes (Table 1). For all the traditionally “aquatic” organisms (flagellates, nematodes, and rotifers) strong-to-moderate correlations were observed (Table 1). Moderate correlation was also observed between hexapodans and mites, but weak or no correlations existed between the other combinations of organisms or pollen (Table 1). Thus, concentrations of traditional aquatic organisms (flagellates, nematodes, and rotifers) similarly vary, while concentrations of the more mobile hexapodans and mites vary similarly with each other.
Table 1. Spearman $\rho$ correlations between mean pollen, flagellate, and invertebrate concentrations per L of stemflow and throughfall. Bolded values indicate significant correlations at $p < 0.05.$

|       | Pollen | Flagellates | Nematodes | Rotifers | Mites | Hexapodans |
|-------|--------|-------------|-----------|----------|-------|------------|
| Pollen |        | **0.43**    | **0.39**  | **0.28** | -0.14 | -0.18      |
| Flagellates |        |             | **0.75**  | **0.48** | 0.03  | 0.03       |
| Nematodes |        |             |           |          | -0.07 | 0.08       |
| Rotifers |        |             |           |          | -0.05 | 0.11       |
| Mites |        |             |           |          |       |            |
| Hexapodans |        |             |           |          |       | **0.47**   |

This is the first study known to the authors to document the concentration of flagellates in throughfall and stemflow. Flagellates could be the most concentrated of observed particulates in this study, especially in stemflow during pollen shedding where the median was $\sim 50 \times 10^6$ cells L$^{-1}$ and could be as high as $120 \times 10^6$ cells L$^{-1}$ (Figure 1b). These flagellate cell concentrations are smaller than bacterial cell concentrations measured in throughfall and stemflow beneath tree canopies via flow cytometry: $\sim 10^7$-$10^9$ cells L$^{-1}$ (Bittar et al., 2018). Still, the transport of $10^5$-$10^7$ flagellate cells L$^{-1}$ storm$^{-1}$ from the phyllosphere (where they have long been known to reside: Ruinen, 1961; Bamforth, 1973; Flues et al., 2018) to spatially-localized soil areas may have ecological relevance. Indeed, in other lotic environments, flagellate concentrations are similar—e.g., in temperate rivers concentrations range from $\sim 1$-$37 \times 10^6$ cells L$^{-1}$ (Basu and Pick, 1997; Karrasch et al., 2001)—and these organisms are considered ecologically relevant at those concentrations, especially in intermittent streams (Romani et al., 2017) which may be a better analogy for throughfall and stemflow. Of the observed flagellate cells, many were photosynthetic. A few were identified as euglenoids; the remainder lacked clear, distinguishing features, though they were most likely chlorophytes, chrysophytes, and/or cryptomonads. All of these taxa have been previously identified as members of phytotelmata communities (Gebühr et al., 2006; Plachno and Wolowski 2008).
Few investigations known to the authors have reported the concentration and flux of nematodes, rotifers and/or mites—both report data for stemflow only, one for common central European tree species under natural rainfall (Ptatscheck et al., 2018) and another for a maize cropland under irrigation (Ellsbury et al., 1996). Thus, this is the first report of these organisms’ concentrations in both throughfall and stemflow. Stemflow was several times more concentrated in nematodes than throughfall (Figure 1c), which is near or within the range of nematode enrichment previously observed. Ellsbury et al. (1996) uniformly applied the entomopathogenic nematode, *Steinernema carpocapsae*, and irrigation to control rootworm and found that both irrigation waters and nematodes were significantly concentrated by stemflow, by 3.9 times and 3.1-4.6 times, respectively, compared to above-canopy application amounts. Ptatscheck et al. (2018) stemflow nematode concentrations from large stemflow-generating tree species (*Carpinus betula* and *Fagus sylvatica*: 10 to >300 nematodes L\(^{-1}\)) compared favorably to our dogfennel stemflow samples, especially when pollen was absent (10 to ~400 nematodes L\(^{-1}\)). They did not provide a comparison of stemflow concentrations to gross rainfall or throughfall; however, they discussed having “collected an exceptionally large number of small juvenile nematodes” (Ptatscheck et al., 2018). Although Ptatscheck et al. (2018) observed greater concentrations of rotifers than nematodes in tree stemflow, rotifer concentrations in dogfennel stemflow were typically half those observed for nematodes—perhaps due to the different growth form/habitat of our understory forb. Mite concentrations were similar between our study and Ptatscheck et al. (2018), which generally ranged from 0-16 mites L\(^{-1}\) v. 0-20 mites L\(^{-1}\), respectively.
This is also the first study known to the authors to document the concentration of hexapodans (beyond collembolans) in throughfall and stemflow. Indeed, despite insects being ubiquitous canopy residents, they are typically perceived as a contaminant (and discarded) in past work: e.g., see methods of Dezzeo and Chacón (2006) and discussion by Ponette-González et al. (2020). Given the total 92.9 L m\(^{-2}\) of net rainfall observed across 7 studied storms, the median of 5 insects L\(^{-1}\) (hexapodans excluding collembolans) across both fluxes results in an estimated input of 465 insects m\(^{-2}\). The insects found in water samples were, of course, corpses, but diverse (including aphids, ants, and beetles). Regarding collembolans, our average stemflow concentrations were similar to, though slightly smaller than, those reported in Ptatscheck (2018) for the smooth bark trees: 4-6 collembolans L\(^{-1}\) (regardless of pollen) for dogfennel stemflow versus 7 collembolans L\(^{-1}\) for *C. betula* and 8 collembolans L\(^{-1}\) for *F. sylvatica* stemflow. The total flux of these organisms is, of course, increased by those transported in throughfall. As these results are similar and ecologically significant across the only observations available for a cropland (Ellsbury et al., 1996), a forest (Ptatscheck et al., 2018), and our understory site, we re-emphasize the call made by these previous studies for greater investigation of rainfall partitions as critical mediators of biotic particulate exchange between the canopy, litter and soils.

**Conclusions.**

For the five storms that occurred during the *Pinus palustris* pollen shedding event at our site, total pollen flux through the *Eupatorium capillifolium* (dogfennel) understory canopy was 95.6 x 10\(^6\) grains ha\(^{-1}\). Although this represents 0.1-3.2 g ha\(^{-1}\) season\(^{-1}\), throughfall and stemflow beneath dogfennel is highly spatially variable (coefficients of variability were 38% and 254% for
throughfall v. stemflow, respectively), which may result in localized particulate inputs. This may especially be true for stemflow, which represented 37% of the total pollen flux and can be spatially concentrated (18-200 times compared to the stand-scale overstory throughfall).

Results suggest that some organisms observed in throughfall and stemflow can be more concentrated during pollen shedding (flagellates, nematodes and rotifers) and others showed no clear change or correlation with pollen (mites and hexapodans). Flagellates and hexapodan concentrations in throughfall and stemflow were reported here for the first time and were found in ecologically relevant quantities. Combined, these biological particulates represent a large flux of materials bringing nutrients to the soil that has barely been studied to-date.

Declarations

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Conflicts of Interest/Competing Interests. The authors declare no conflict of interest.

Availability of Data/Code. Data are available in the supplemental materials and anything additional can be provided by request from corresponding author. No code used in this work.

Authors’ contributions. MG and JTVS conceived and designed the study in consultation with DARG, to complement his hydrometeorological research. DARG designed/deployed field collection devices, collected samples, and analyzed the hydrological data in consultation with JTVS. MG processed samples, performed the microscopy, and analyzed the particulate data. JTVS drafted the initial article with input from all authors. All authors contributed to the manuscript writing.

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