Expansion of the Pollination Service Measurement (PSM) concept in Southern Ontario

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Executive Summary

- Animal pollinators play a central role in sustainability of both plant populations, and of the ecosystems functions that they mediate.
- This document will expand the earlier proof-of-concept study to include a broader range of plant species in terms of flowering time and pollinator specificity, additional study sites, and a wider range of expected pollination service.
- Following on the success of the purplestem aster (*Symphyotrichum puniceum*) in measuring pollination last year, Pollination Canada coordinated the "Purplestem Aster Pollination Adventure" (PAPA) in the Kitchener-Waterloo area. Preliminary results show promise as a tool of pollination service evaluation, and as an education and outreach tool in pollinator conservation.
- The lack of seed set in the Control flowers of all species that successfully set seed indicates that they were self-incompatible. Although not statistically significant, *S. pilosum* showed some production of seeds in control branches. It is not known whether or not the fruits contained viable seed or were parthenocarpic fruits.
- Seed set in Treatment flowers was not statistically correlated with available pollinator assemblage data, but largely reflected expectations of pollination service at the study sites. Ranked seed set values at the study sites were consistent across all six species of *Symphyotrichum*, indicating the value of these metrics in pollination service assessment (PSM).

Introduction

Flowering plants form the trophic basis of productivity in most terrestrial ecosystems, and the maintenance and sustainability of plant populations, independent of human intervention, is crucial for the sustainability of the ecosystems themselves. Animal pollinators, including but not limited to bees, flies, butterflies, beetles, bats, and birds, play a vital role in mediating sexual reproduction of approximately 85% of the world's flowering plants, and 78% in temperate regions such as Canada (Ollerton et al. 2011). While pollination in agricultural systems can be improved relatively easily through the use of honey bees or other managed pollinators, such approaches to management of natural ecosystems are neither economically nor logistically feasible (Mader et al. 2010). Conservation of native, wild pollinators is thus critical to ensuring reproductive success and biodiversity of plant species (Fontaine et al. 2006; Ollerton et al. 2011). The first step to ensuring that pollinators provide sufficient pollination is monitoring pollinators and pollination success in both agricultural and non-agricultural landscape elements.

Historically, biomonitoring methods have relied on structural variables measured for some portion of the biotic community, such as plants, fish or aquatic invertebrates (Karr 1981; Allan et al. 1997; Townsend et al. 1997), that act as a proxy measurement for ecosystem services or processes, or an (often poorly defined) concept of ecosystem "health" or "integrity" (Karr 1981; Karr 1992). The use of community structure of organisms to infer the rate or quality of the ecosystem process that they perform is a common approach to assessing ecosystem health, but can have mixed or unpredictable results (Schwartz et al. 2000). Evaluation of communities, and by inference the provision of ecosystem services, using these methods (richness, diversity, abundance, other community metrics) has significant drawbacks, namely high labour requirements for field sampling and sample processing, requirement for expensive taxonomic expertise, and high variability in the resulting data that makes interpretation and action difficult. Conceptualization may also be difficult for most people, who remain unaware of Canada's bee diversity (>900 native species), let alone the species of flies, moths, beetles, and other insects that provide ecosystem pollination services. In recent years, the development of approaches that directly measure ecosystem function has been encouraged, although none have been explicitly developed for the function of pollination in a monitoring context.

Evaluation of plant reproductive success using ambient vegetation or potted plant phytometers has been used successfully to address a variety of ecological questions related to

pollination. Seed set in ambient vegetation has been used to examine landscape-level pollination service and competition among plants for pollinators (Dauber et al. 2010; Trant et al. 2010; Hennig & Ghazoul 2011). Potted plants have been used to measure pollen limitation (Campbell 1985; McKinney & Goodell 2010), effects of neighbouring blooms on plant reproductive success (Kunin 1997; Bosch & Waser 2001; Schulke & Waser 2001; Spigler and Chang 2009), pollination responses to agricultural practices (Brittain et al. 2010a,b), and pollinator habitat responses (Steffen-Dewenter et al. 2002; Artz & Waddington 2006; Sperling & Lortie 2010). The Pollination Service Measurement (PSM) system described here directly measures pollination service at a site by evaluating plant reproductive success (seed set) in a standard array of potted plants. This has the advantages of directly measuring the target ecosystem service rather than inferring it from pollinator assemblage data, and requires less time and technical expertise (and therefore incur significantly lower costs) than surveys of pollinator assemblages. By measuring seed set in available blossoms over a defined time period, pollination success can be quantified.

This document will expand the earlier proof-of-concept study that evaluated the potential of three species of fall asters (*Symphyotrichum*) to include a broader range of plant species in terms of flowering time and pollinator specificity, additional study sites, and a wider range of expected pollination service. The major issue in previous studies was the delayed bloom in the greenhouse grown plants, which was particularly troublesome in *S. cordifolium*, and likely related to the light "pollution" from neighbouring greenhouses interfering with the flowering phenology of these short-day plants. This issue has been addressed by moving to a greenhouse that receives ambient light only. While some data describing health of the pollinator assemblages will be available for examination in the context of PSM at most of the sites used this year, this will be de-emphasized in favour of the PSM metric (seed set) itself.

Methods

Study Sites

The following twelve sites in southern Ontario were selected for this study, in the Carolinian, Great Lakes, and St. Lawrence Forest ecoregion. Early plans for sites in the Boreal Shield ecoregion were found to not be feasible due to logistical issues. The first five sites form a pollination gradient and were used in the previous study of *Symphyotrichum* species.

- Eastview (EAS) is a decommissioned landfill in Guelph, Ontario (43.577N, 80.232W). The landfill was capped in the early 1990s, and overseeded with a grass mix. Since that time, it has developed a community of plants dominated by non-native species. Pollinator monitoring from 2009-2011 indicated very low pollinator abundance, particularly for bees.
- Waynco (WAY) is a decommissioned gravel pit located south of Cambridge, Ontario (43.328N, 80.300W). The site is intended for rehabilitation, but the current vegetation has regenerated without human intervention. Pollinator monitoring from 2009-2011 indicated high abundance and diversity of bee and syrphid species.
- Blair Flats East (BFE) is located at the Rare Charitable Research Reserve in Blair, Ontario (43.385N, 80.367W). It is a former agricultural field (corn/soybean rotation) that has been left to regenerate without human intervention since its final harvest in 2009. Pollinator monitoring in 2010 and 2011 indicates a flower visitation rate approximately 25-35% that of the other sites at Rare (CCF and GSF, see below), which were similar to each other. Pollinator sampling occurred in 2010-2012.
- Cruickston Creek Field (CCF) is located at the Rare Charitable Research Reserve in Blair, Ontario (43.377N, 80.351W). It is a former agricultural field (corn/soybean rotation) that has been left to regenerate without human intervention since its final harvest in 2003. Pollinator sampling occurred in 2010-2012.
- George Street Field (GSF) is located at the Rare Charitable Research Reserve in Blair, Ontario (43.377N, 80.341W). It is a former agricultural field (corn/soybean rotation) that has been left to regenerate without human intervention since its final harvest in 2006. Pollinator sampling occurred in 2010-2012.
- Townsend House (TSH) is the apiary and honey bee research laboratory at the University of Guelph (43.536N, 80.214W). The test plants were placed approximately 10m from approximately 20 honey bee hives, intended to represent the maximum achievable pollination in the field.
- Farm conservation projects (GIL, LEN). The Norfolk Alternative Land Use Services (ALUS) is a successful program in Norfolk County, Ontario "providing payments to farmers for returning marginal, environmentally sensitive, or inefficient farmland into native vegetative cover and wetlands" (www.norfolkalus.com). Two pairs of sites (with pollinator monitoring data from 2011 and 2012) were selected. Each pair consists of a conservation site

and a comparable nearby unamended site. GIL sites include a hedgerow project modified for pollinator conservation (GIL-A, planted wildflowers, nesting amendments) and an unmodified cedar (*Thuja occidentalis*) hedgerow typical of the area (GIL-N). LEN sites consist of a prairie restored from field crop use (LEN-A), and a grassy, unmodified area at the margin of an unimproved soybean field (LEN-N). Per conditions of our permission to conduct research at ALUS sites, exact locations and names of property owners are not included in any written material.

- Cherryvale Farm (CVF) is a large organic and permaculture farm located in Cherry Valley, Prince Edward County, Ontario (43.933N, 77.147W). Test plants were placed on the farm at two locations (CVF1, CVF2) corresponding with pollinator monitoring sites from 2011 and 2012. Different areas of the farm could potentially have different pollinator communities depending on the available crops.

Throughout this report, "site" refers to one of the study locations detailed above, "plot" refers to one of the [pan trap plus Malaise trap] locations within each of those sites.

Test Plant Species

- All plants were obtained in 72-plug trays from St. Williams Ecology Center in Walsingham, Ontario. This source was chosen because seed is collected from wild populations, and thus it is not expected to have issues of self-incompatibility that may be associated with some nursery stock, in that the seeds were not collected from only a few parents. Seed collected from wild populations can reasonably be expected to have similar relatedness levels as that population.
- Tests were conducted using nine plant species native to southern Ontario. Eight species were placed at all sites, *Symphyotrichum cordifolium* was not placed at CVF sites due to limited availability in 2012:
    - Repeated tests with the three species used in 2011 (*S. puniceum, S. ericoides, S. cordifolium*) at the expanded list of sites. In 2011 flowering was somewhat delayed in *S. ericoides* and highly delayed in *S. cordifolium*. It is suspected that "light pollution" from neighbouring greenhouses interfered with the flowering phenology of these short-day plants. All plants were raised this year in a

greenhouse that receives ambient light only, and issues with delayed bloom or flower buds that refused to open were not detected.

- Because the results with *Symphyotrichum* were encouraging, three additional species in the same genus were also tested this year (*S. oolentangiensis, S. pilosum*). All *Symphyotrichum* species are considered late-blooming generalist plants, and provide significant resources in late summer and fall to a wide variety of pollinators. Due to the error that many of our purchased *S. puniceum* in 2012 were in fact New England aster (*S. novae-angliae*), this species was also tested at all sites except CVF. Many of the seedlings distributed to participants in the citizen science study (see below) were also *S. novae-angliae*. It is suspected that as many as three or four of the plug trays purchased in 2012 were in fact *S. novae-angliae.*
- Two earlier-blooming generalist species of Asteraceae have been selected for investigation, *Helenium autumnale* and *Eupatorium perfoliatum*.
- Two species that are considered pollinator specialists (*Chelone glabra* and *Gentiana andrewsii*) were included in the evaluation. Both species provide abundant rewards that are generally only accessible to large, strong bees such as bumble bees and some leaf-cutter and carpenter bees that are able to force their way into the flowers. These species may also be subject to nectar-robbing, in which flower visitors chew through the tissue near the bottom of the corolla to access the nectar, and thus do not come into contact with the reproductive structures.

Field Procedure

Plants were transplanted from the plug trays to individual 15cm plastic pots, using a standard potting mix for all species. Plants remained in the greenhouse until just before flowering, at which time two stems on each test plant were bagged with Pollinator Exclusion Bags (PEBs), with one tagged as a Control (CON; remain bagged). Any open blooms were removed prior to bagging. Groups of six plants were randomly assigned to each test site, watered well, and placed in a group at a point near the middle of the site in full sun. Grouping of the plants is necessary to ensure that there is a source of pollen in habitats where wild individuals do

not occur. PEBs were then removed from the Treatment (TRT) stems only, control stems remaining bagged throughout. After seven days (more for some species at CVF), the Treatment stems were re-bagged, and plants were watered and returned to the greenhouse. PEBs remained in place until seeds were set.

Herbivory has been a minor issue at some sites in the past, and affected the data collection significantly only once; even fewer issues were noted in 2012, with occasional plants damaged by groundhogs but few lost entirely. However, protection of potted plants from desiccation was not an issue in 2011 experiments with *Symphyotrichum*, but was a problem in 2012, particularly for those plants that bloomed earlier in the season. Table 1 summarizes the deployment dates for all plant species at all sites.

Greenhouse & Laboratory Procedure

Plants in the greenhouse were watered three times per week before and after deployment in the field, using raw well water. Following seed set, flower heads were harvested and returned to the laboratory where seeds were enumerated using a microscope. As in 2011, number of seeds set per flower or inflorescence on the Treatment stems was used as a response variable that can be related to pollinator community characteristics at the study sites. Self-incompatibility of the test species was confirmed by evaluating seed set on the Control stems.

Table 1. Summary of 2012 deployment dates for all plant species at the study sites. "-" indicates that plants were not deployed at those sites due to limited availability, "X" indicates that the plants died at that site, most frequently due to drought conditions prevalent last summer, although *Symphyotrichum ericoides* died for unknown reasons at three sites.

Site	New England aster (*S. novae-angliae*)	Sky-blue aster (*S. oolentangiense*)	Purplestem aster (*S. puniceum*)	Heath aster (*S. ericoides*)	Hairy aster (*S. pilosum*)	Heart-leaf aster (*S. cordifolium*)
EAS	9/11-18	9/11-18	9/18-25	9/18-25	9/25-10/2	9/25-10/2
WAY	9/11-18	9/11-18	9/18-25	9/18-25	9/25-10/2	9/25-10/2
BFE	9/11-18	9/11-18	9/18-25	X	9/25-10/2	9/25-10/2
GSF	9/11-18	9/11-18	9/18-25	X	9/25-10/2	9/25-10/2
CCF	9/11-18	9/11-18	9/18-25	9/18-25	9/25-10/2	9/25-10/2
TSH	9/11-18	9/11-18	9/18-25	9/18-25	9/25-10/2	9/25-10/2
CVF-1	-	9/11-18	9/18-25	9/18-25	9/18-25	-
CVF-2	-	9/11-18	9/18-25	X	9/18-25	-
LEN-N	9/12-19	9/12-19	9/19-26	9/19-26	9/26-10/3	9/26-10/3
LEN-A	9/12-19	9/12-19	9/19-26	9/19-26	9/26-10/3	9/26-10/3
GIL-N	9/12-19	9/12-19	9/19-26	9/19-26	9/26-10/3	9/26-10/3
GIL-A	9/12-19	9/12-19	9/19-26	9/19-26	9/26-10/3	9/26-10/3

Site	Common boneset (*Eupatorium perfoliatum*)	Turtlehead (*Chelone glabra*)	Closed gentian (*Gentiana andrewsii*)	Sneezeweed (*Helenium autumnale*)
EAS	X	X	8/28-9/4	X
WAY	X	X	8/28-9/4	X
BFE	X	X	8/28-9/4	X
GSF	X	X	8/28-9/4	X
CCF	X	X	8/28-9/4	X
TSH	X	X	8/28-9/4	X
CVF-1	7/19-26	X	9/3-11	X
CVF-2	7/19-26	X	9/3-11	X
LEN-N	-	-	-	-
LEN-A	-	-	-	-
GIL-N	-	-	-	-
GIL-A	-	-	-	-

Citizen Science Pilot Program

Following on the success of the purplestem aster (*Symphyotrichum puniceum*) in measuring pollination last year, Pollination Canada coordinated the "Purplestem Aster Pollination Adventure" (PAPA) in the Kitchener-Waterloo area, which was marketed as an educational and citizen science program to existing members of Pollination Canada and Seeds of Diversity, and through venues such as local food markets. There are some drawbacks to this method compared to the potted plant approach, including variations in water availability, soil conditions, and the potential for human error. The type of information this project yielded is different from the experimental approach, but in addition to its educational and outreach value it has the potential to provide information about PSM across broad areas of the landscape, both

urban and rural. It will also educate and engage the general public about the value of pollination and native plant species in general, and the concerns about reproduction of plants in particular, including PSM.

Twenty-five participants in Waterloo Region were recruited and provided with six *S. puniceum* plants each. These plants were planted in a single patch in each participant's home garden, with instructions to plant in full sun if possible, and to provide "one full watering can twice a week, regardless of rainfall". Following seed set but prior to seed shedding, participants estimated the total number of inflorescences on each plant, harvested five mature seed heads from each plant and submitted them for seed enumeration. Participants were provided with addressed envelopes, each containing six smaller envelopes (one per plant) to return the seed heads to Pollination Canada. As mentioned above, a considerable number of the provided plants were actually *S. novae-angliae* due to supplier error. The two species were treated separately in the analyses.

Pollinator Sampling

Pollinator assemblage characteristic measurement is de-emphasized in the current project, in favour of sampling more sites and plant species to develop the PSM concept. However, pollinator sampling occurred in 2012 at all sites except EAS and WAY. Insects were collected in Malaise traps and coloured pan traps (blue, yellow) (Figure 1). Two major groups of pollinators (bees, syrphid flies) form the basis of the pollinator assemblage descriptors for the sites. The three sites at the Rare Charitable Research Reserve (BFE, CCF, GSF) each had one Malaise trap and eight permanent sample plots, each containing one yellow and one blue pan trap. The four ALUS sites (LEN-A, LEN-N, GIL-A, GIL-N) and the two CVF sites (CVF-1, CVF-2) were sampled approximately monthly using one Malaise trap and eight pan traps per site by personnel from Norfolk County and Cherryvale Farm, respectively. Sampling equipment was deployed for 48 hours approximately once per month during the growing season, in good flying weather for pollinators whenever possible. In order to compare trap catches across the sites for the purposes of this study, a "standard unit" (SU) consisting of [eight blue plus eight yellow pan traps] plus one Malaise trap per sampling date is used.

Statistical Analyses

Self-incompatability of each species was confirmed using a simple t-test comparing seeds per flower on TRT vs. CON branches. An Analysis of Variance (ANOVA) of seed set on TRT branches for each test species was conducted to assess differences between sites, using the mean number of seeds per flower on each of the experimental plants. Thus, each plant is an experimental unit (not each flower), and n=6 at each site. For those plants that were deployed longer than seven days at Cherryvale Farm (see Table 1), the seeds per flower is multiplied by [7/(#days deployed)] to standardize deployment time.

Quantitative results from pan and Malaise traps are used in the analysis, standardized to number per Malaise trap per sampling period per site to produce the insect catches per SU described above. Abundance (n), taxa richness (R), and Shannon-Wiener diversity (H') were calculated at three different temporal scales: all available SUs at the site (2010-2012), all SUs in 2012 only, and the SU in August 2012 (i.e. the closest to the time the *Symphyotrichum* test plants were deployed at the sites). 2012 samples were not available for EAS or WAY, so these sites were excluded from the latter two calculations. ANOVA was performed to compare n, R, and H' at the study sites using all available samples. Seed set (seeds per flower) from TRT branches in the test species was related to pollinator assemblage metrics [abundance, taxa richness, Shannon-Wiener diversity (H')] at the three temporal scales described using univariate regression, with the site as the experimental unit.

In the Citizen Science Pilot Program, mean seed set per flower was calculated for all submitted material from the participants. Using ArcGIS 9.3, addresses were plotted on a map and land coverages calculated using data available from the Grand River Conservation Authority. Coverages were calculated within a 100m radius of each participant, representing the maximum expected foraging distance for pollinators, and 200m radius, representing the broader landscape. Proportional land use for each was divided into four categories: Residential, Commercial-Industrial, Agricultural, and "Green", which included parks, golf courses, and uncultivated rural lands and woodlots. Correlations between these coverages and seed set per flower were calculated.

Figure 1. Malaise trap (top) and yellow pan trap (bottom).

Results & Discussion

Seed Set

Three of the nine original plant species deployed failed to set seed and/or died in the field, likely due to the high temperatures and drought in July and August 2012. Thus, *Chelone glabra*, *Eupatorium maculatum*, and *Helenium autumnale* are excluded from the results. The heat and drought issues that occurred in summer 2012 also caused noticeable senescence of wild plants at many of the study sites. Although many of the study plants recovered and produced new foliage after returning to the greenhouse and being watered, all the flowers were destroyed and no seeds were produced. In future, drought and heat tolerance will need to play a larger role in selection of species for testing. While drought-stressed plants are expected to show reduced attractiveness to pollinators, decreased pollen viability, decreased seed set and increased abortion rates, and decreased seed mass for a variety of reasons (Kjohl et al. 2011, and references therein). Effects in this study are much more likely to be related to effects on pollinators, which are poorly studied; most of the research in this area has been conducted on wind-pollinated plants, notably corn. For example, many solitary bees prefer hot, dry sites for nesting, although certain behaviours such as foraging and interactions with flowers can be affected by weather conditions, and affect pollination activity. Surface waters were not available within 100m of the plants at most sites in either year (there are wetlands near BFE and CVF sites). Considerable work remains to be done on relationships of climate and weather with pollinator survival and behaviour (Kjohl et al. 2011).

Gentiana andrewsii survived its deployment in the field, but pollination was practically zero at all sites except CVF-1, which was particularly unexpected, due to the very low seed set of other species at this site (see below). As anticipated, individual flowers showed either very low seed set (0-2 seeds, possibly due to pollen delivery by ovipositing moths), or very high seed set (200 seeds or more in four flowers at CVF-1). The identity of the pollinator at CVF-1 is unknown. We intend to experimentally measure pollination efficacy of bumble bees on this species in 2013, and observe wild pollinators visiting the flowers in the field, if possible. Thus, it may be practical and efficient in future to enumerate *G. andrewsii* flowers pollinated rather than counting all seeds as a measure of PSM.

The lack of significant seed set in the control branches of all species that successfully set seed (Figure 2) indicates that they were indeed self-incompatible, despite being monoecious, and

therefore were suitable choices as phytometers for this experiment (t-tests by species, all $p<0.01$). Typical of the Asteraceae, the florets at the edge of each individual inflorescence become receptive first, and the disc florets undergo anthesis, releasing pollen to the top of the floret, followed a day or two later by the opening of the stigmatic lobes to expose the receptive surface. This proceeds in concentric rings until the central disc florets bloom a few days after the peripheral florets. Full pollination of an inflorescence would therefore require multiple visits by pollinators over the period of bloom (Chmielewski & Semple 2001, 2003; Woodcock et al. 2012). *Gentiana andrewsii*, selected because of its potential to evaluate pollination by large bees such as bumble bees, has many potential seeds per flower but appears to have "all or nothing" pollination, in that seeds were set in very few flowers, and only flowers at one site, but those flowers had many filled seeds. The *G. andrewsii* plants deployed at CVF-1 (the site that had pollination of this species) had about one-third of flowers with set seed, and an average of 164 seeds per flower. Seeds were present only in the Treatment flowers. *Symphyotrichum novae-angliae*, due to the unexpected abundance of plants as explained in the Methods, was added to the study, bringing the total number of plant species for which results are presented to six, all in genus *Symphyotrichum*.

For the sites at which sampling was repeated in 2012, seed set per inflorescence was comparable to that observed in 2011 for *S. cordifolium*, *S. puniceum*, and *S. ericoides* (Woodcock et al. 2012). As in 2011, variance in seed set for all species was high (Woodcock et al. 2012). Analyses of Variance (ANOVA) of seeds per inflorescence on the treatment branches for each species revealed no statistically significant differences between the sites for *S. oolentangiense*, *S. puniceum*, or *S. pilosum* (Figure 2). The remaining three species did have differences between the highest and lowest seed set values. Despite the high variance and minor differences between species, a wide range of responses were observed (Figure 2), and results ranked by site were similar across the species (Table 2). When the species were ranked according to seed set per inflorescence, the sites with the highest seed set were generally the Norfolk County sites (both ALUS and NON-ALUS), and the sites at the Rare Charitable Research Reserve, particularly CCF. CVF-1, EAS, TSH, and WAY were lowest. The results at WAY were unexpected, since seed set and the pollinator assemblage were strong there in 2011 (Woodcock et al. 2012). However, as the site is an abandoned gravel pit with poor soil, the drought of summer 2012 may have negatively impacted pollinators. The low seed set at TSH suggests that

honey bees do not forage readily on *Symphyotrichum* flowers, and activity of other pollinators in proximity to numerous honey bee hives is low.

Table 2. Summary of ranked seed set per inflorescence, from low (1) to high (9-12, depending on plant species) for each test plant species. "ND" indicates that plant species were not deployed at that site, "L" indicates those lost in the field due to herbivory or other mortality. "Corrected Sum" corrects the sum of the raw ranks for those sites at which all plant species were not deployed.

SITE	*S. cordifolium*	*S. ericoides*	*S. puniceum*	*S. novae-angliae*	*S. oolentangiense*	*S. pilosum*	**Sum Ranks**	Corrected Sum
CVF1	ND	2	1	ND	2	2	**7**	11
EAS	3	3	5	5	1	7	**24**	22
TSH	1	1	6	1	7	8	**24**	22
WAY	1	5	7	6	3	6	**28**	26
BFE	5	L	1	4	4	10	**24**	29
GSF	7	L	4	2	12	4	**29**	35
GILN	4	6	11	3	8	5	**37**	35
CVF2	ND	L	9	ND	5	L	**14**	42
LENN	9	7	10	9	6	3	**44**	42
CCF	6	4	8	7	10	9	**44**	42
GILA	8	9	12	8	9	1	**47**	45
LENA	10	8	L	10	11	11	**51**	55

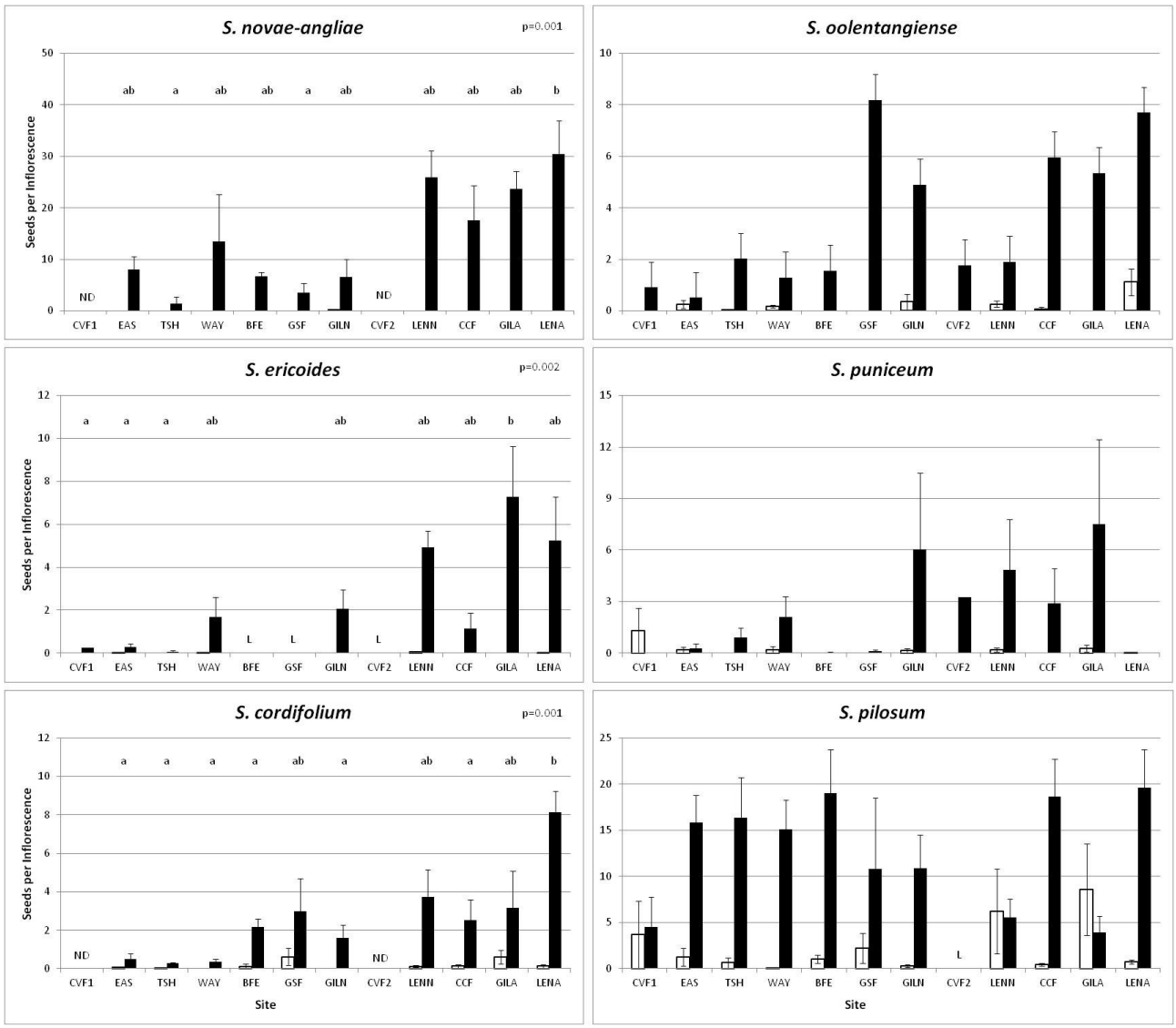

Figure 2. Seed set of six species of *Symphyotrichum* at the study sites (Control = white bars, Treatment = black bars). "ND" indicates that plant species were not deployed at that site, "L" indicates those lost in the field due to herbivory or other mortality. Bars sharing the same letter code are not significantly different from one another (GLM, Tukey test, α=0.05). Error bars show 1 S.E.

Pollinator Assemblages

All three assemblage metrics (n, R, H') showed high variance, but statistically significant differences were detected among the sites in richness ($p<0.001$) and H' ($p<0.01$). Pollinator abundance was not different among sites due to high variability, although mean abundance per plot varied threefold among the sites. Abundance, richness, and H' were highly autocorrelated, particularly within temporal scale of measurement, and ANOVAs are reported using all available samples, since this most closely represents the season-long pollination service characteristics that are the target for evaluation. Lists of bee (Table 3) and syrphid (Table 4) taxa recovered at each site, by all trapping methods and over all seasons (2009-2012) are provided, with exact interval varying by site (see Methods). These numbers are not directly comparable due to the different sampling efforts between the sites. On a per-sample basis, richness was significantly greater at WAY than CVF2, GIL-A, or LEN-A, and H' was greater at WAY and CCF than at CVF-2 (Figure 3). No other differences were statistically significant in these descriptors. There were no statistically significant relationships between seed set on pollinator assemblage characteristics for any of the test species.

Table 3. Occurrence of bee (Apoidea) taxa at the study sites. P = kleptoparasitic bee.

Species	BFE	CCF	EAS	GSF	WAY	GIL-N	GIL-A	LEN-N	LEN-A	CVF-1	CVF-2
Agapostemon sp.	X	X		X	X	X	X	X	X	X	
Andrena sp.	X	X	X	X	X	X		X	X	X	X
Anthidiellum notatum		X									
Anthidium manicatum		X		X	X						
Anthophora sp.				X						X	
Apis mellifera	X	X	X	X	X	X			X		X
Augochlorella aurata	X	X	X	X	X	X	X	X	X	X	X
Bombus bimaculatus	X	X		X	X						
Bombus borealis		X	X		X						
Bombus griseocollis			X								
Bombus impatiens	X	X	X	X	X	X	X		X		
Bombus perplexus	X										
Bombus rufocinctus	X	X	X		X						
Bombus sandersoni					X						
Bombus vagans	X	X				X	X	X		X	
Calliopsis andreniformis					X	X					
Ceratina sp.	X	X	X	X	X	X	X	X	X	X	X
Coelioxys octodentata					P						
Coelioxys rufitarsus		P	P	P	P						
Coelioxys sayi							X				
Colletes eulophi		X		X							
Colletes hyalinus	X										
Colletes mandibularis	X										
Colletes nudus	X										
Colletes simulans	X			X							
Dufourea sp.	X	X	X	X	X						
Epeolus sp.	P										
Halictus confusus	X	X	X	X	X	X	X			X	
Halictus ligatus	X	X	X	X	X	X	X	X	X	X	X
Halictus rubicundus	X	X	X	X	X		X				
Heriades sp.		X			X						
Hoplitis sp.	X	X	X	X	X						X
Hylaeus sp.	X	X	X	X	X					X	X
Lasioglossum sp.	X	X	X	X	X	X	X	X	X	X	X
Megachile sp.	X	X	X	X	X			X	X	X	X
Melissodes sp.	X	X	X	X	X			X	X		X
Nomada sp.	P	P	P	P	P	P				P	
Osmia conjuncta	X		X	X	X						
Osmia caerulescens		X	X								
Osmia atriventris					X						
Osmia distincta						X				X	
Osmia proxima		X							X		
Osmia pumila				X						X	
Osmia simillima			X		X		X		X	X	
Peponapis pruinosa									X		
Perdita octomaculata	X										
Protandrena sp.	X	X		X		X					
Sphecodes sp.	P	P	P	P	P	P	P	P	P	P	
Stelis lateralis	P			P	P						
Triepeolus sp.					P						
Xylocopa virginica	X	X	X	X	X						
Parasitic Bee Richness	**5**	**3**	**3**	**4**	**6**	**2**	**1**	**1**	**1**	**2**	**0**
Total Bee Richness	**31**	**30**	**24**	**28**	**32**	**15**	**12**	**10**	**14**	**16**	**10**

Table 4. Occurrence of flower fly (Syrphidae) taxa at the study sites.

Species	BFE	CCF	EAS	GSF	WAY	GIL-N	GIL-A	LEN-N	LEN-A	CVF-1	CVF-2
Allograpta micrura				X							
Allograpta obliqua	X		X	X	X					X	
Chalcosyrphus metallicus										X	
Chalcosyrphus nemorum	X	X		X	X	X		X		X	
Chrysotoxum pubescens		X		X	X				X		
Epistrophe nitidicolis	X										
Eristalinus aeneus		X			X						
Eristalis anthophorina		X									
Eristalis arbustorum	X	X		X							
Eristalis dimidiata	X		X		X						
Eristalis flavipes	X	X		X							
Eristalis stipator				X							
Eristalis tenax	X	X	X		X						
Eristalis transversa	X	X									
Eumerus sp.		X		X				X	X		
Eupeodes americanus	X		X		X				X		
Eupeodes pomus			X		X						
Eupeodes sp.	X	X		X		X		X			
Eupeodes volucris		X									
Eurosta solidagnis	X										
Ferdinandea buccata					X						
Helophilus fasciatus	X	X	X								
Heringia salax		X									
Heringia sp.										X	
Lejops sp.			X							X	
Limenitis archippus		X									
Mallota posticata		X									
Melanostoma mellinum		X	X	X	X						
Merodon equestris			X	X							
Microdon tristis		X									
Ocyptamus fascipennis		X	X								
Orthonevra nitida		X									
Paragus haemorrhous				X							
Paragus sp.		X		X	X		X	X	X		
Parhelophilus laetus		X	X								
Platycheirus angustatus			X							X	
Platycheirus hyperboreus			X	X	X	X		X		X	
Platycheirus nearcticus			X		X						
Platycheirus obscurus			X							X	
Platycheirus quadratus	X		X		X	X				X	X
Platycheirus scambus		X	X			X				X	
Platycheirus sp.		X									
Sphaerophoria asymmetrica				X							
Sphaerophoria bifurcata		X									
Sphaerophoria brevipilosa				X							
Sphaerophoria contigua	X	X	X	X	X					X	
Sphaerophoria philanthus	X	X	X	X	X			X			
Sphaerophoria sp.	X	X	X			X	X	X			X
Sphegina petiolata		X									
Spilomyia longicornis	X			X							
Syritta pipiens	X	X		X							
Syrphus rectus	X										
Syrphus ribesii	X		X								
Toxomerus geminatus	X	X	X	X	X	X		X	X	X	
Toxomerus marginatus	X	X	X	X	X	X	X	X	X	X	X
Trichopsomyia apisaon						X					
Tropidia quadrata						X					
Xylota quadrimaculata		X	X								
Total Syrphid Richness	**22**	**32**	**24**	**22**	**18**	**10**	**3**	**9**	**6**	**13**	**3**
Bee + Syrphid Richness	**53**	**62**	**48**	**60**	**50**	**25**	**15**	**19**	**20**	**29**	**13**

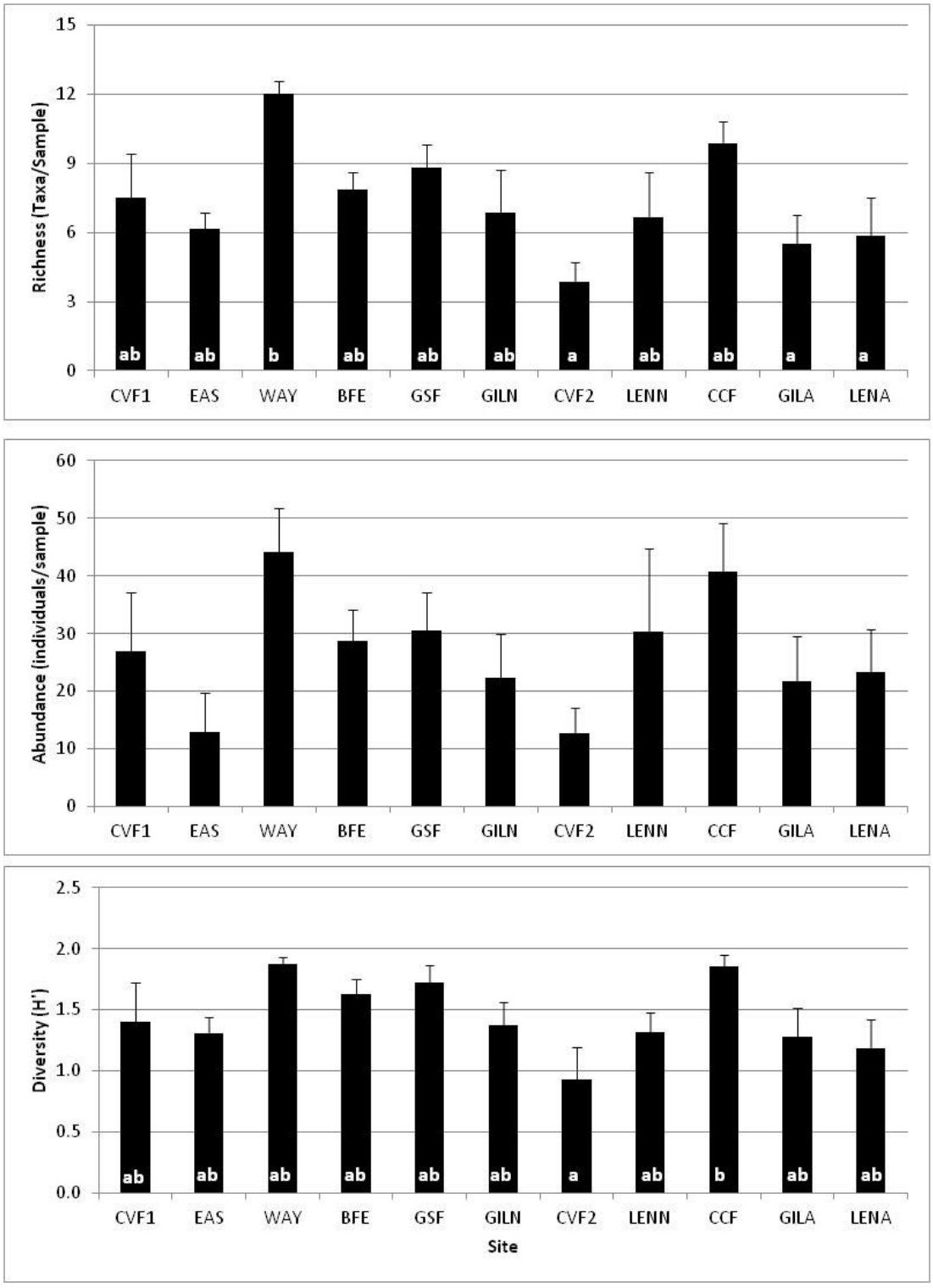

Figure 3. Basic community metrics (top to bottom - Richness, Abundance, Shannon-Wiener Diversity) of Apoidea and Syrphidae at the study sites. Values are given as the mean per plot across all sampling events, 2009-2012. Bars sharing the same letter code are not significantly different from one another (GLM, Tukey test, α=0.05). Error bars show 1 S.E.

Citizen Science Pilot Program

Fourteen of 25 original participants submitted seeds for counting, and of these seven submitted seed heads from fewer than the six plants that they were originally given. Many of the participants had plants that died or did not flower, possibly due to insufficient watering under the difficult conditions in summer 2012. Also, seed heads from 45 of the plants returned by participants were actually New England asters rather than purplestem asters, which had seed heads from only 20 plants returned (see Methods for explanation). The species will be discussed separately.

Most of the participants lived in built-up, residential areas, although all except two had some parkland or other greenspace within 200m of their home (Figure 4). A wide range of responses were observed in terms of mean seed set per participant, with *Symphyotrichum puniceum* ranging between 0.1-14.8 mean filled seeds per inflorescence and *S. novae-angliae* ranging between 4.6-94.2 mean filled seeds per inflorescence. Seed set in *S. puniceum* responded positively to residential land use, but negatively to Commercial-Industrial land. Seed set in *S. novae-angliae* showed the opposite pattern (Table 5). Somewhat surprisingly, "Green" space had weak correlation with seed set at either scale. Horn (2010) found that Industrial and "old" Residential lands (established neighbourhoods with little soil disturbance, established tree canopies and gardens) supported fairly strong urban pollinator assemblages. Although the sample size of this pilot study is small and potential sources of variation numerous, the correlations with land use and pollinator assemblage characteristics described in Horn (2010) suggest scientific value in this approach, in addition to value in public engagement and education.

Table 5. Correlation coefficients (Pearson r) between seed set and proportional land cover for the PAPA pilot project for *Symphyotrichum puniceum* and *S. novae-angliae*. at 100m and 200m radius. Agriculture land use is omitted, as it was only present at one participant site.

	100m radius			200m radius		
	Residential	Commercial -Industrial	"Green"	Residential	Commercial -Industrial	"Green"
S. puniceum	0.452	-0.339	0.035	0.525	-0.331	-0.208
S. novae-angliae	-0.308	0.302	0.237	-0.002	0.365	-0.128

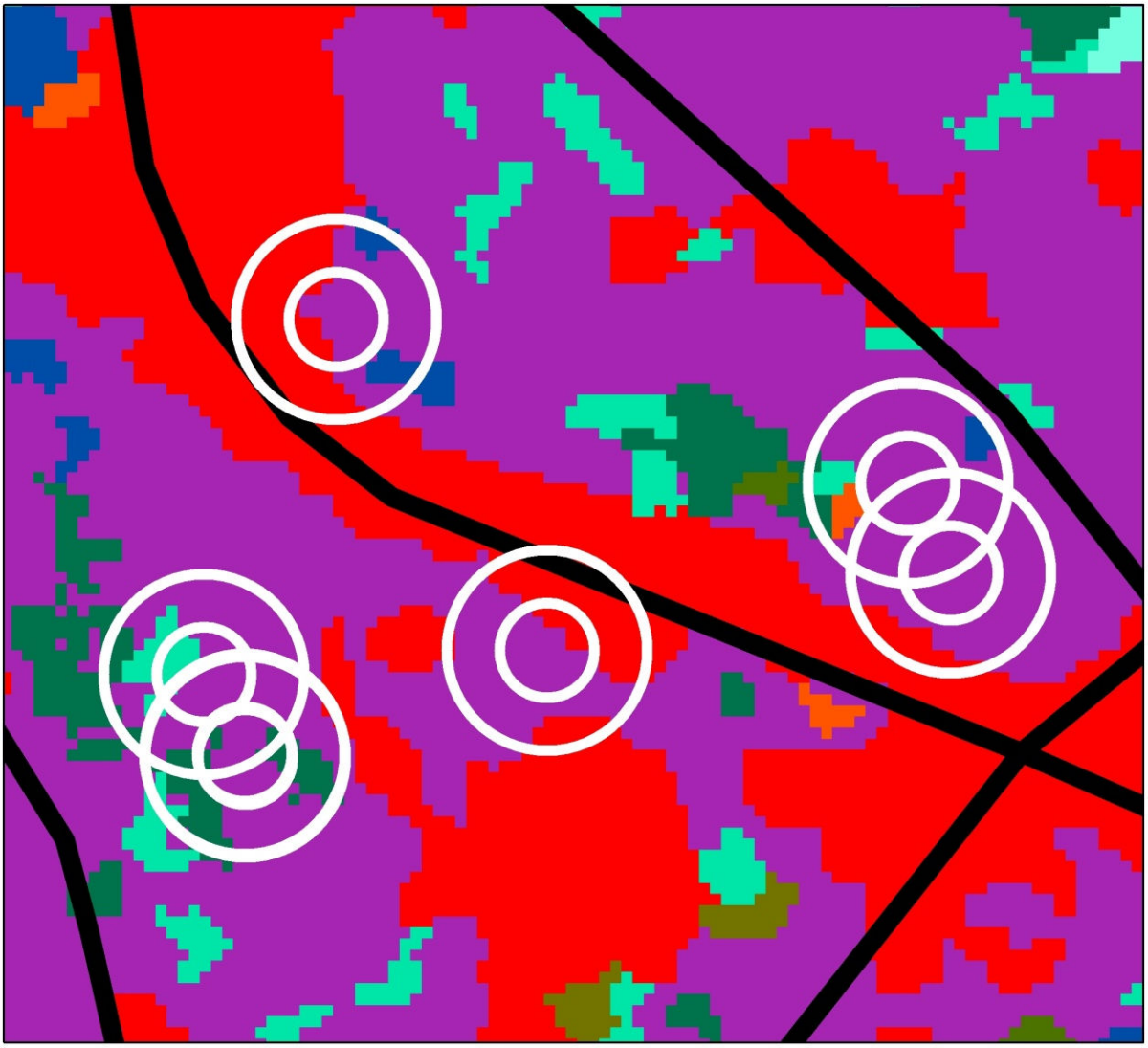

Figure 4. A cluster of PAPA participant sites near downtown Waterloo, with coverages showing Residential (purple), Commercial-Industrial (red) and "Green" (various green and blue shades) land uses. The inner white circle at each point shows a 100m radius centered on the participant, the outer circle a 200m radius. Only main roads (black lines) are shown.

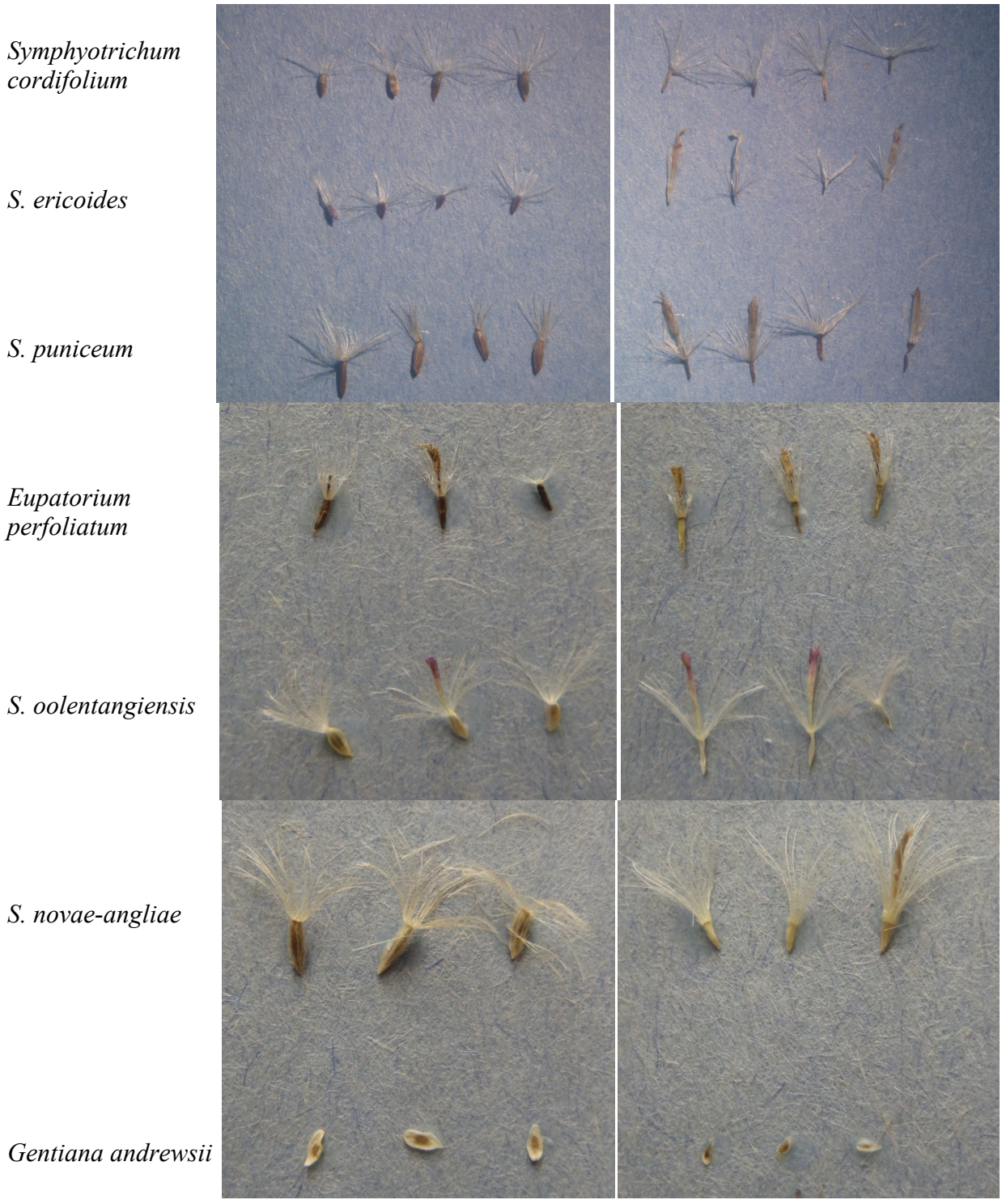

Figure 5. Seed photos. Filled seeds are on the left panel for each species, unfilled seeds on the right. Seeds for *Symphyotrichum pilosum, Helenium autumnale,* and *Chelone glabra* not shown.

Concluding Remarks

The PSM system continues to show promise for evaluating pollination success directly, rather than inferring it from pollinator collections. The system has been demonstrated to be less costly in terms of both money and labour than systems that rely on pollinator community sampling. This method does not require specialized knowledge or skill to execute, and fruits containing filled seeds for all species are easily distinguished from those containing unfertilized seeds (examples given in Figure 5). Problems associated with greenhouse light pollution that were experienced in 2011 were apparently solved in 2012 by moving plant culture to a greenhouse that received only ambient light. Furthermore, herbivory was a minor mortality source in the field. However, in future an increase in sample size to eight or even ten plants per site may be warranted, possibly divided into two groups a standard distance apart (e.g. 20m or 50m). The efforts to include plants specifically to evaluate certain components of the pollinator assemblages (i.e. large vs. small bees, generalists vs. specialists, long- vs. short- corolla pollinators, and so forth) was a failure, largely due to choice of plants that were unsuited to exposure in pots in the field, particularly under the hot, dry conditions experienced in southern Ontario in summer 2012.

Pollinator assemblage sampling was limited in the 2012 project. However, unlike the 2011 sampling, in 2012 the assemblage descriptors (n, R, H') were not significantly related to seed set in any of the six species tested. All species showed a broad range of response, and a similar ranking of sites that indicates the success of the approach. Apart from unexpected results at WAY (low seed set), and to some degree the low seed set at TSH, the PSM results approximated expectations and further demonstrated the value of this approach, despite the lack of statistically significant correlation with pollinator assemblage metrics. As in 2011, it should be noted that the complexity of analysis was considerably less than would have been the case for pollinator assemblage sampling, and differences between sites likely no easier to detect.

The Citizen Science Pilot Project (PAPA) showed promise in evaluating pollination service, although further investigation is necessary into the relationships between the measurements and the numerous factors, including pollination, that relate to reproductive success. Furthermore, simplification of plant care is necessary, so that response rate improves, particularly during difficult years such as 2012. It may be worth considering using a more

drought-tolerant plant that can better survive neglect, such as *S. ericoides*. However, the enthusiasm and engagement of the participants was undeniable.

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Appendix. PSM scores (seed set) for the six test species of *Symphyotrichum*, expressed as seed set per flower per 7-day period. Variability is expressed as standard error (SE), given in parentheses. Sites are ranked from apparent lowest to highest overall pollination service (see Table 2).

Site	*S. cordifolium*	*S. ericoides*	*S. puniceum*	*S. novae-angliae*	*S. oolentangiense*	*S. pilosum*
CVF1	-	0.24(0)	-	-	0.91(0.72)	4.49(3.27)
EAS	0.51(0.28)	0.28(0.14)	0.27(0.27)	8.06(2.47)	0.51(0.31)	15.81(2.95)
TSH	0.26(0.04)	0.06(0.06)	0.90(0.56)	1.33(1.33)	2.02(1.63)	16.35(4.40)
WAY	0.33(0.14)	1.67(0.94)	2.10(1.20)	13.46(9.14)	1.29(0.88)	15.10(3.20)
BFE	2.16(0.43)	-	0.04(0.04)	6.70(0.72)	1.55(0.70)	19.00(4.76)
GSF	2.99(1.70)	-	0.10(0.08)	3.50(1.89)	8.18(1.48)	10.80(7.70)
GILN	1.59(0.69)	2.04(0.89)	6.02(4.46)	6.54(3.48)	4.89(2.40)	10.86(3.63)
CVF2	-	-	3.24(0)	-	1.77(1.65)	-
LENN	3.74(1.42)	4.92(0.78)	4.84(2.93)	25.85(5.23)	1.91(0.68)	5.52(2.05)
CCF	2.51(1.09)	1.14(0.74)	2.89(2.03)	17.51(6.83)	5.96(0.77)	18.64(4.09)
GILA	3.14(1.93)	7.28(2.35)	7.51(4.93)	23.68(3.44)	5.34(1.70)	3.92(1.75)
LENA	8.13(1.09)	5.23(2.04)	-	30.43(6.53)	7.69(2.67)	19.58(4.18)