

Pollen sleuthing for terrestrial plant surveys: Locating plant populations by exploiting pollen movement

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PREMISE OF THE STUDY: We present an innovative technique for sampling, identifying, and locating plant populations that release pollen, without extensive ground surveys. This method (1) samples pollen at random locations within the target species' habitat, (2) detects species' presence using morphological pollen analysis, and (3) uses kriging to predict likely locations of populations to focus future search efforts.

METHODS: To demonstrate, we applied the pollen sleuthing system to search for artificially constructed populations of *Brassica rapa* in an old field. Population size varied from 0–100 flowers labeled with artificial pollen (paint pellets). After characterizing the landscape, we pan-trapped 2762 potential insect vectors from random locations across the field and washed particulate matter from their bodies to assess artificial pollen abundance with a microscope.

RESULTS: Population size greatly influenced artificial pollen detection success; following random pollen trap sampling and interpolation, ground surveys would be best focused on identified areas with high pollen density and low variation in pollen density. Sampling sites most successfully detected artificial pollen when they were located at higher elevations, near showy flowering plants that were not grasses.

DISCUSSION: Detection of nascent populations using the proposed system is possible but accuracy will depend on local environmental factors (e.g., wind, elevation). Conservation and invasive species control programs may be improved by using this approach.

KEY WORDS detectability; invasive species monitoring; plant conservation; pollen dispersal; spatial interpolation; survey methodology.

Informed management and policy decisions, especially those attempting to control invasive pests or recover endangered species, require accurate knowledge of individual location and abundance. However, many species are not easily or consistently detectable; indeed, there is a non-trivial probability that target species will remain undetected during a survey (e.g., Kery, 2002; Wintle et al., 2005; Garrard et al., 2015). The consequences of false absences during weed surveys may be especially severe because invasive plants often go undetected during a substantial lag phase common during early invasion (Kowarik, 1995; Crooks and Soule, 1999; Radosovich, 2002). Yet, the lag phase of a nascent invasion or satellite population is expected to be the easiest to control (Moody and Mack, 1988; Zamora and Thill, 1999; Sakai et al., 2001). Therefore, research should address imperfect detectability and develop new measures for avoiding high false absence rates. We propose an alternative survey method that addresses problems of detection and identification that contribute to uncertainty associated with detec-

tion inaccuracy. The proposed method provides an approach to detect and locate nascent, invading populations using pollen samples collected in traps.

Information about plant population distributions and abundances comes from many sources, including formal survey work by scientists, happenstance reports by citizen scientists, and indigenous and traditional knowledge (Turner et al., 2000; Beckie et al., 2003; Silvertown, 2009). The design of formal survey strategies depends on focal species, resident landscape, habitat information, and resource constraints. As a result, a number of techniques are employed and can include comprehensive surveys, quadrat-based surveys, targeted surveys within predictor habitats (e.g., point or line transects, radial searches), and remote sensing based on detectable plant characteristics such as flowering color (Hill et al., 2005; Rew et al., 2006; Crall et al., 2013). With the exception of remotely sensed data, human surveyors must conduct laborious searches, often traversing large areas of land.

Terrestrial angiosperms and conifers periodically release pollen, and this biological process can be exploited to increase survey area and search efficiency. Pollen grain movement through space can be modeled based on physical characteristics of both the particles and environment (Niklas, 1985; Beckie and Hall, 2008). Using estimates of settling velocities and likely settlement locations, researchers may calculate a pollen dispersal curve, which describes a frequency distribution of dispersal distances based on both species-specific pollen traits and weather (Okubo and Levin, 1989; Klein et al., 2003). Generally, at a landscape scale, pollen movement may be characterized by large concentrations near the release site, which decrease exponentially with increasing distance from the source population (Campbell, 1991; Folloni et al., 2012). Yet, pollen dispersal curves are species- and environment-specific (Jarosz et al., 2003; Hofmann et al., 2010). The pollen vector involved (i.e., wind, animal, water) can also influence the distance traveled and the pollen settlement pattern (Rader et al., 2011). Thus, knowledge of pollen dispersal patterns across landscapes may aid in locating likely source locations, as long as pollen can be reliably identified (Erdtman, 1943; de Nascimento et al., 2009).

When pollen vectors are sampled across a landscape (and at least some pollen is captured), spatial interpolation can be used to estimate the likely location of the source, accounting for environmental variation that may affect pollen movement. This mapping process can be automated such that spatial interpolation is based on acceptable default settings (Hiemstra et al., 2009). Pollen capture may depend on the vector, source population size and density, sampling distance, weather conditions, and the type of landscape. Larger populations should release larger clouds of pollen and therefore be easier to detect (Campbell, 1991). They may also be detectable at a greater distance from their source than small populations (MacArthur and Wilson, 1967; Handel, 1983; Ellstrand, 1992). Similarly, environmental conditions such as wind velocity, elevation, and rainfall at sampling sites could influence the distance and amount of transported pollen. Finally, areas that attract vectors may act as better sampling locations than areas that lack attractants (e.g., nectar-consuming insects may be attracted to high densities of floral resources; Pyke, 1984).

Here, we propose a new approach to locate plant populations (and, more broadly, any population that releases vector-borne pollen) (Fig. 1). To focus search efforts, digital image analysis or even

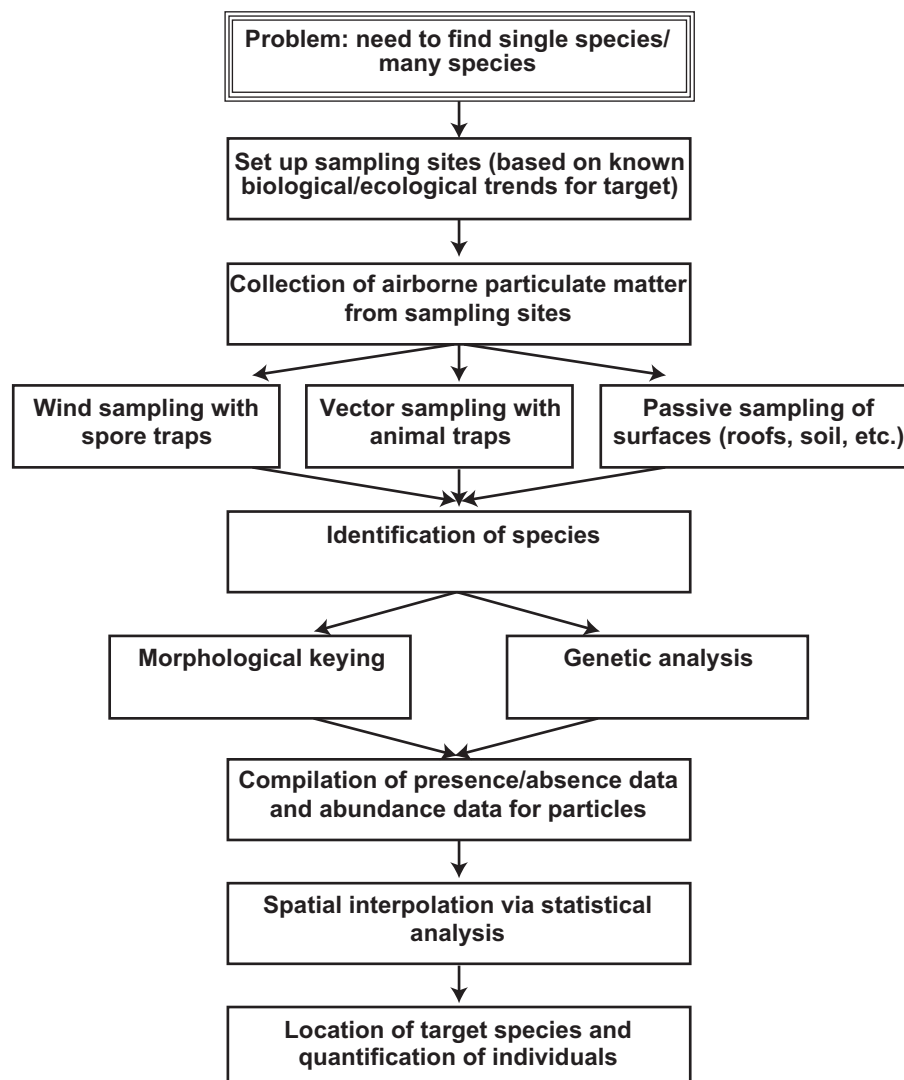


FIGURE 1. Schematic diagram of the pollen sleuthing system proposed to collect, identify, and locate the source of pollen within a natural landscape.

genetic technology can then be used to facilitate species identification, and automatic interpolation can be used to predict the likely locations of source populations. Furthermore, quantitative data on pollen abundance may predict demographic characteristics of focal populations. Notably, our identification, location, and monitoring system may be useful for finding rare biological populations within species-diverse, complex, outdoor environments (Fig. 1). To determine whether a pollen sleuthing system could be used to detect source population locations, we used experimental populations of *Brassica rapa* L. that vary in size. Furthermore, we explored whether our pollen sleuthing system could direct us to plant populations of interest by labeling the flowers of our experimental *B. rapa* populations with artificial pollen (paint pellets), and then sampling the landscape for artificial pollen. We evaluated the accuracy of this system by locating our study populations within a natural, complex landscape using spatially explicit data obtained from captured artificial pollen grains. We address the following questions:

1. To what degree can location of a source population be predicted?
2. Does the floral abundance (pollen and individual abundance) at source populations influence our ability to detect their location?
3. Do environmental conditions influence our ability to predict the location of a source population?

METHODS

Study system

Brassica rapa (Brassicaceae), or field mustard, is a fast-growing Eurasian annual herb introduced to North America as either a seed contaminant or crop escape (Frankton and Mulligan, 1987; Rollins, 1993; Darbyshire, 2003). When found outside of agricultural fields, *B. rapa* grows in a patchy distribution, and occurs in ruderal habitats, generally in open grasslands and on roadside verges (Britton and Brown, 1897; Robinson and Fernald, 1908; Gulden et al., 2008). Plants have pale yellow flowers arranged in dense clusters at the top of highly branched stems (Gulden et al., 2008). It is often used as a model plant in ecological and evolutionary studies because of its hardy nature, long flowering season, and an abundance of available genetic information (Williams and Hill, 1986; Cheng et al., 2011). *Brassica rapa* is an obligate outcrossing species (Takayama et al., 2000), and pollen movement is accomplished by both wind and insect vectors. In the closely related *B. napus* L., reproduction is largely effected by insect pollinators and influenced by wind direction (Mesquida and Renard, 1982; Beckie et al., 2003). Syrphid flies, native bees, and *Pieris* butterflies were common flower visitors observed on our plants.

Study site

Koffler Scientific Reserve (KSR) at King City, Ontario, Canada, is a 350-ha research area that includes open, reclaimed pasture, small buildings, and protected old-growth forest within the Oak Ridges Moraine (elevational range: 278–318 m above sea level). Typical of moraines, the terrain varies considerably over a small spatial scale and is therefore a perfect location for investigating the potential of our approach. In the study area, pan-trap sampling efforts covered an area of approximately 1 km × 1.5 km (Fig. 2). Naturalized populations of *B. rapa* were not detected, allowing us to experimentally imitate a spatially rare organism by creating artificial populations in pots (Blaney, 1999). We also repeatedly searched the landscape

visually for *B. rapa* using both walking and driving surveys, but we did not detect any naturalized populations during the season under investigation. Detailed maps of topography, vegetation, and soil conditions in the study area are available in Blaney (1999) and from the Koffler Scientific Reserve (University of Toronto). During the time when we collected data, the average air temperature ranged from 25–36°C, wind speeds varied from 0.5–2 m/s, and wind direction ranged from 111–226.5°.

Field experiment

Seeds were provided by E. Austen (Austen and Weis, 2015). Seeds were planted on 15 June 2013 in seedling trays containing standard potting soil (PRO-MIX BX peat; Premier Tech, Rivière-du-Loup, Québec, Canada) and watered regularly. Individuals were reared under hoop-house conditions and planted 25–29 June 2013 at the seedling stage in Cone-tainers (Stuewe and Sons, Corvallis, Oregon, USA) so that we could easily vary population size and randomize individuals. Between 16 August and 11 September 2013, we constructed a single outdoor population of *B. rapa* to simulate a rare population of plants. Number of individuals and number of flowers within populations were manipulated (Table 1, location described in Fig. 2). To prevent contamination and ensure simplicity in this preliminary study, only one model population was present in the landscape at one time. After flowering (16 August), the experimental population was created in an open field (in a single location) by placing an appropriate number of potted individuals (see below) in a tray placed within a children's play pool with the bottoms of Cone-tainers submerged in water. Unused individuals remained outside the hoop house (44°01'45.84"N, 79°32'20.64"W) covered by pollinator exclusion bags (DelStar pollinator exclusion bag, 750- μ m gauge; DelStar, Austin, Texas, USA) to reduce movement of pollen from dehiscing flowers.

On evenings (between 1500–1700 hours) prior to designated sampling dates, we arranged experimental populations such that they represented one of three population size treatments: large (100 individuals), medium (50 individuals), or small (five individuals). After setting up the experimental population each day, anthers of one flower from every plant (100, 50, or five) were painted with as much artificial pollen as would stick. We used fluorescent yellow paint pellets (4.5 μ m in diameter) as artificial pollen grains, which are comparable in size to maize pollen grains. Paint pellets appear as powder to the naked eye and are comparable in color to *B. rapa* pollen. We expected artificial pollen grains to be transported easily by insect vectors (Fenster et al., 1996; Van Rossum et al., 2011), although there are some applications for which this would not have been appropriate (e.g., bird-pollinated plants; Waser, 1988). For reference, *B. rapa* pollen grains are less than 22.8 μ m long (Crompton and Wojtas, 1993), which may mean that artificial pollen grains could potentially travel differently than *B. rapa* pollen. Importantly, because we did not count the number of paint pellets applied to flowers, we cannot relate either pellet abundance to the abundance of real pollen nor do we know how standardized the amount of pellets applied to the flowers were (which may influence the accuracy of population size estimates from pellet density).

Treatments were randomly imposed so that the three population size treatment levels occurred once over a three-day sampling period, and we attempted to replicate each treatment level three times between 16 August and 11 September 2013 to minimize seasonal variation in pollinators, individual plant size, and weather conditions (Table 1). A control population was set up on three additional

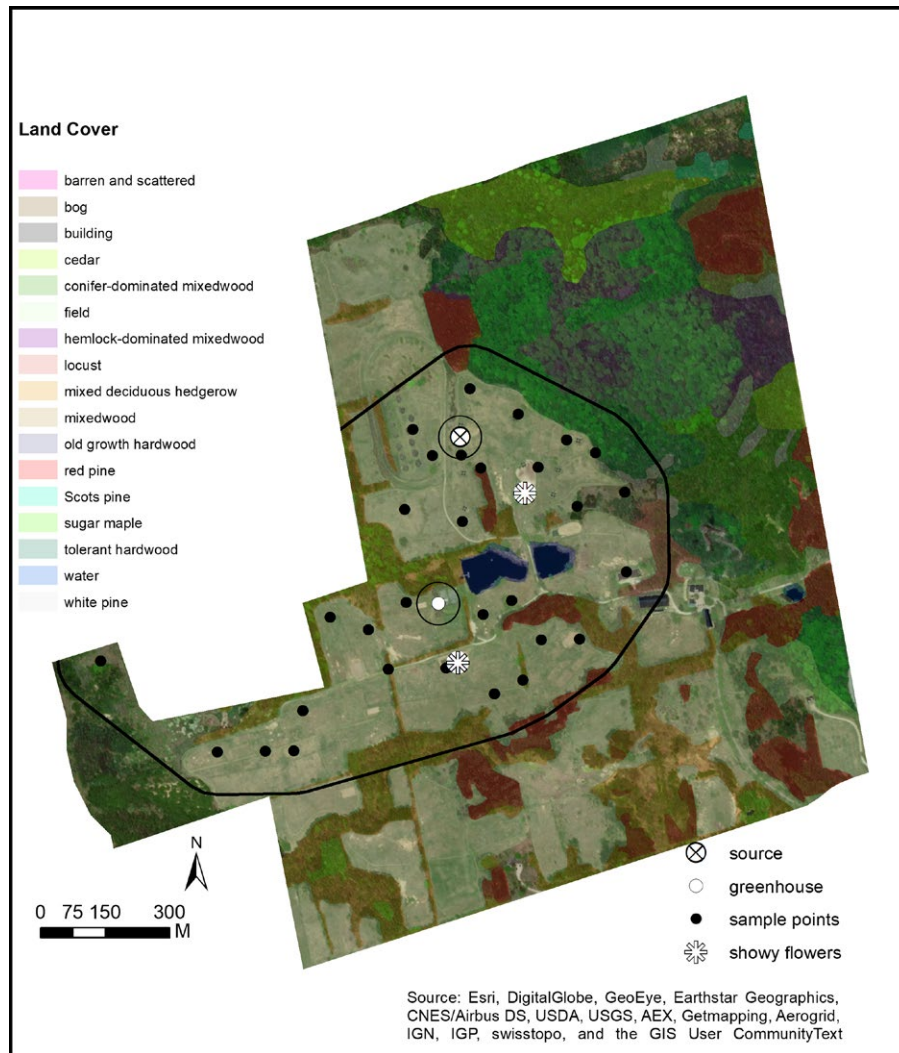


FIGURE 2. Study area and location of pollen sample pan traps (sample points) at the Koffler Scientific Reserve in King City, Ontario, Canada. The source pollen location at $44^{\circ}01'58.33''\text{N}$, $79^{\circ}32'18.04''\text{W}$ is circled and depicted by an 'X'. A potential pollen source at $44^{\circ}01'45.84''\text{N}$, $79^{\circ}32'20.64''\text{W}$ where extra plants were stored at the greenhouse is also depicted (open circle). Locations of showy, flowering, non-experimental plant populations are identified with an asterisk. Distance values in the scale bar are given in meters.

days. In the control population, all plants were covered with pollen exclusion bags, making population size for this treatment equal to zero individuals (Table 1). This experimental setup was not performed on days that received rainfall (or when rain was predicted), given that we expected artificial pollen movement via wind or insects to be relatively low under rainy conditions.

Sample collection and preparation for identification

We collected artificial pollen samples at 30 randomly chosen locations at KSR (Appendix 1). The same randomly selected sampling sites were applied to all treatments. To sample the artificial pollen load carried by insects (and capture windborne artificial pollen movement), we used pan traps, which capture a wide diversity of insects, including potential pollinators of *B. rapa*. We set out one solid white plastic bowl (10 cm in length by 6 cm in width) on the ground as a trap in each sampling location for 24 h (Dollar Store, Newmarket, Ontario, Canada). The traps were nestled among plants and half-filled with soapy water to trap insects (Dawn liquid dish soap,

Proctor & Gamble, Toronto, Ontario, Canada; Droege, 2009). At the end of 24 h, pan traps were emptied into 50-mL tubes, washed, and replaced at all locations.

Samples were then prepared for analysis roughly following the wash method (Shoemaker, 2010). From each pan trap, all insects were placed in plastic 50-mL centrifuge tubes (no. 525-0216; VWR, Radnor, Pennsylvania, USA), and leftover trap water was collected in a second sample tube and frozen at -20°C until further processing. Both samples were thawed, and artificial pollen loads were collected from each insect specimen by vortexing for approximately 30 s and inverting sample tubes four times until a considerable portion of particulate matter disconnected from insect bodies and floated in the surrounding liquid (the liquid appeared cloudy). Scopa were removed from collected specimens, dissected to increase the likelihood that all pollen collected by bees would be potentially evaluated, rather than hidden in a scopa, and then placed in the sample tube. Insects were then removed from the tubes, and the artificial pollen load solutions were centrifuged at 17,000 rpm for 2 min. The

TABLE 1. Schedule of experimental treatments and subsequent pollen sleuthing sampling events imposed.^a

Sampling event	Date	Population size	No. of point samples
1	16 August	100	25
	17 August	50	22
	18 August	5	24
	19 August	0	27
2	20 August	50	24
	21 August	50 ^b	28
	22 August	5	22
	23 August	0	16
3	24 August	5	28
	9 September	100	25
	10 September	50	26
	11 September	0	17

^aThe experimental populations of *Brassica rapa* were created by assembling populations that varied from 0, 5, 50, or 100 labeled flowers at the Koffler Scientific Reserve in King City, Ontario, Canada. We trapped potential insect pollen vectors in 16–28 pan traps randomly scattered across an old field landscape between 16 August and 11 September 2013.

^bThe population size on this date was meant to be large (i.e., 100 plants), but only consisted of 50 because many plants failed to flower on that date.

supernatant was drained, and the pellet was smeared on a microscope slide. All insects were identified to family (www.Bugguide.net). On 46 of 360 sampling events (12.8%), the sample was lost. We suspect wild turkeys inadvertently upset the pan traps.

Data collection

Throughout the season, we recorded environmental and biotic variables associated with field site, sampling locations, and location of the source population. Weather data were recorded daily from a single weather station on the KSR property (including air temperature, wind direction and speed, and total precipitation). At each sampling site, we recorded elevation, distance from source population, and surrounding dominant vegetation. The latter estimate was based on a brief visual survey of the presence or absence of plants having showy flowers within 10 m of the sampling point. Whereas in most search scenarios, the distance between a sampling site and the source population would be unknown, in our artificial scenario, we used this distance to the source population measure to identify the maximum distance between sampling locations that could lead to source population detection.

Data recorded from the experimental population included total number of flowering plants and total number of flowers. We also recorded data from our pan traps that provided information on (biotic) insect vector diversity and abundance (i.e., number of species, total abundance of all insects trapped, and abundance of bees, which are some of the more effective biotic pollen vectors in old field communities), as well as amount of artificial pollen captured. Because we were not able to count all of the artificial pollen that was centrifuged off insect vectors, we sampled artificial pollen loads from each pan trap by counting the number of fluorescent paint particles in a single transect through the middle of a prepared microscope slide at 40× magnification (total area sampled: 25 mm × 5 mm or 125 mm²).

Data analysis

To determine the location of source populations, recent developments in the geostatistical literature allow spatial interpolation in an automatic mapping context using acceptable default settings (Hiemstra et al., 2009). We used an R package to perform this task

(R package *automap*; Hiemstra, 2013). Spatial interpolation by kriging using *automap* allows the user to create continuous (mapped) predictions of dependent variables across the study area from point-based sample estimates. The resultant map (i.e., of artificial pollen abundance in this case) describes where artificial pollen density was highest, given the sampling design. With kriging, predictions are associated with prediction errors, and prediction errors can be used to hone in on areas where uncertainty is high—or low—as the case may be (e.g., Melles et al., 2011). For example, kriging prediction errors can be used to optimize spatial sampling designs by minimizing mean prediction errors across a study area (Brus and Heuvelink, 2007; Melles et al., 2011). Therefore, this technique is suitable for predicting likely pollen sources. In the context of locating rare plant populations or early stages of a plant invasion, kriging prediction errors of pollen grain abundance can provide an effective measure of spatial accuracy. This method assumes, of course, that the model is correct and that measurement error is reasonably low.

We determined the maximum artificial pollen abundance detected at each sampling location (i.e., maximum of the three replicate treatments) for each of the three experimental populations and the control (0, 5, 50, and 100). We used maximum pollen abundance per trap for interpolation as this was deemed a more representative measure than a mean or a minimum. The nature of samples in our pollen traps meant that there were many zeroes in these data. When pollinators were present, pollen abundance was high, but if they were absent, pollen abundance decreased to zero or close to zero in many cases. Maximum pollen abundance is a better measure in such cases than the mean (or minimum) because the mean would underestimate pollen by including those days when we did not capture any pollinators in a pan trap.

Maximum artificial pollen abundance for each of these treatments was interpolated by ordinary kriging to create a spatially continuous map of predicted abundance of artificial pollen. We used categories of pollen pellet abundance (e.g., Fig. 3) to allow for an immediate determination of where large differences in pollen amounts occur in the landscape. Category breaks were selected for even spacing, but natural breaks or deviations from mean values could be used. Mapping by interpolation relies on a model of spatial structure—a variogram—to depict how pollen abundance varies with increasing distance between sampling locations. The variogram is akin to a dispersal kernel. Variograms are commonly used to model spatial structure in the field of geostatistics, although they are less commonly used in the context of pollen movement. Dispersal kernels typically show an expected decrease in pollen abundance with increasing distance from source plants, which may be exponential or more gradual. This decrease is captured by the gradual rise to the sill (i.e., the upper limit of the variogram) in the exponential or spherical variogram model. The variogram sill, a measure of average variation, was calculated as the mean squared difference in pollen abundance between pairs of sampling points, which are separated by a given distance class. The sill is known in geostatistics as the level at which near points are no longer more similar than distant points, and the range is the distance at which the model variogram reaches the sill. Akin to a dispersal kernel, the range provides an unbiased estimate of the distance at which the dispersal kernel tail levels off.

To determine what conditions influenced detectability, we first analyzed the variance in the abundance of artificial paint pellets captured at each sampling location. Initially, we ran a general linear model, assuming a Poisson distribution (for artificial pollen count

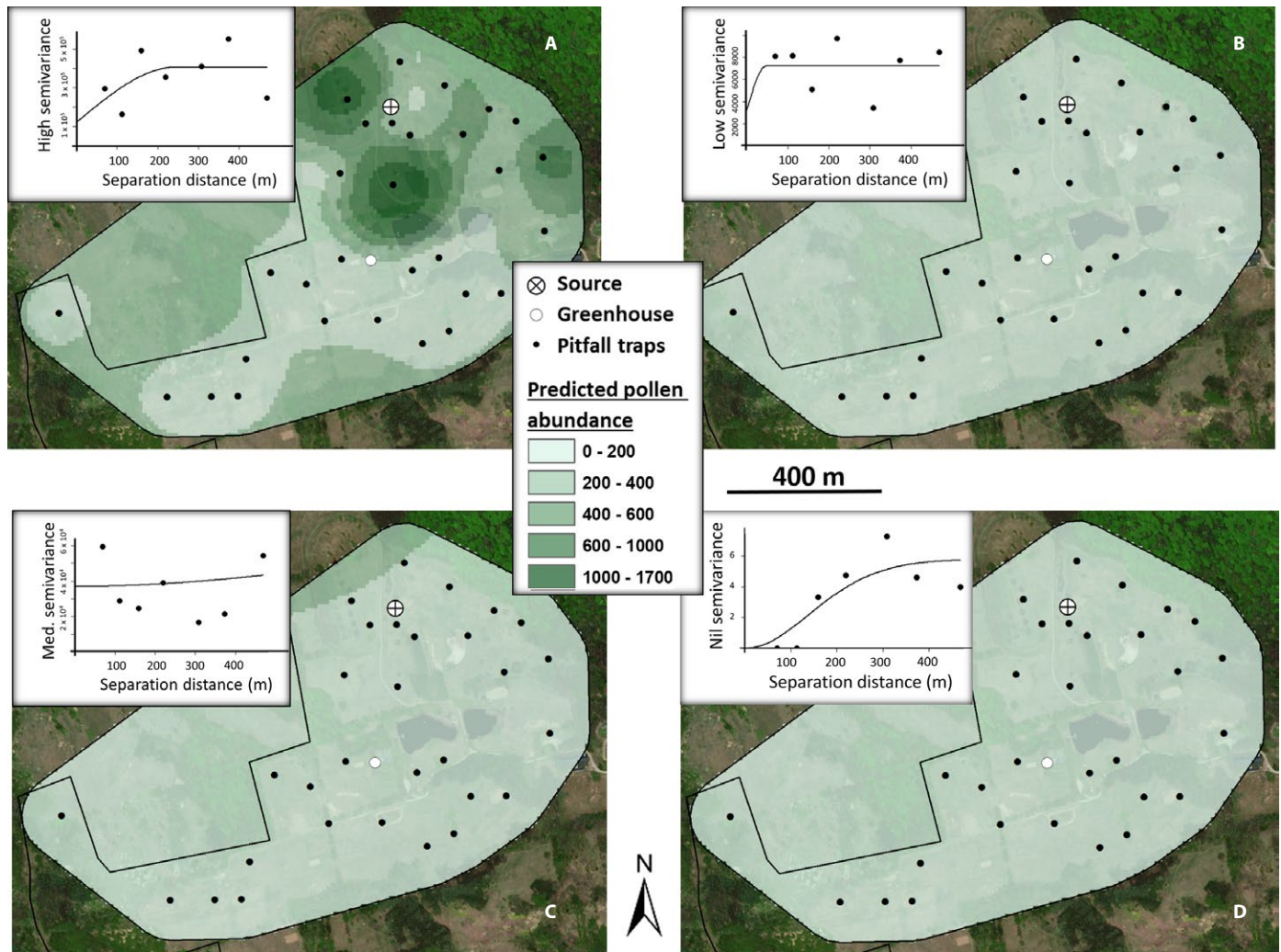


FIGURE 3. Predicted number of pollen grains (by interpolation) based on randomly sampled point locations ($n = 30$ pan traps used to estimate pollen) distributed throughout the field at Koffler Scientific Reserve, King City, Ontario, Canada. Each map depicts pollen predicted based on experimental treatments of artificial pollen grains applied to 100 individual flowers (A), 50 individual flowers (B), five individual flowers (C), and a control (D). Locations of source and potential pollen escape at the greenhouse are depicted using the same symbols as in Fig. 2. Category breaks were selected for even spacing. Each panel contains a variogram (akin to a dispersal kernel) depicting how pollen abundance varies with increasing distance between sampling locations.

data) with three independent variables (one fixed effect [experimental population size] and two random effects [dominant vegetation and sample replicate ($n = 3$)] and covariates of elevation and number of bees collected in the sample. We used PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, North Carolina, USA) to analyze the relationship between artificial pollen and experimental population size, elevation, presence/absence of showy flowers in the vicinity, and number of insects caught in a sample (Poisson distribution, logit link function). Because we were limited in the number of degrees of freedom in the model, we removed factor interactions with replicates from the model.

RESULTS

We captured 2762 insects in total over the experiment, these included a significant number of small native bees or wasps, flies, and

beetles, and fewer than 65 bumble bees, honey bees, and butterflies. Ants, dragonflies, and spiders were extremely rare. A total of 15,652 pollen grains were counted, and samples contained between zero and 2396 paint pellets (mean = 55 pellets, SD = 220). We created maps of paint pellet density that were predicted using ordinary kriging for each experimental population size by modeling spatial structure in artificial pollen grains detected across all pan traps. The experimental treatment with a single source population of 100 labeled flowers resulted in high amounts of spatial structure that could be easily modeled using automated mapping techniques. The variogram inset (Fig. 3A) depicts how neighboring samples are more similar than distant ones, and this spatial structure was modeled using an exponential variogram curve. Results of predictions based on variogram models of spatial structure are depicted in the form of continuous maps of predicted number of paint pellets (artificial pollen) across the sampled area (Fig. 3A–D). At the 100-flower experimental population size, predicted pollen was highest at and

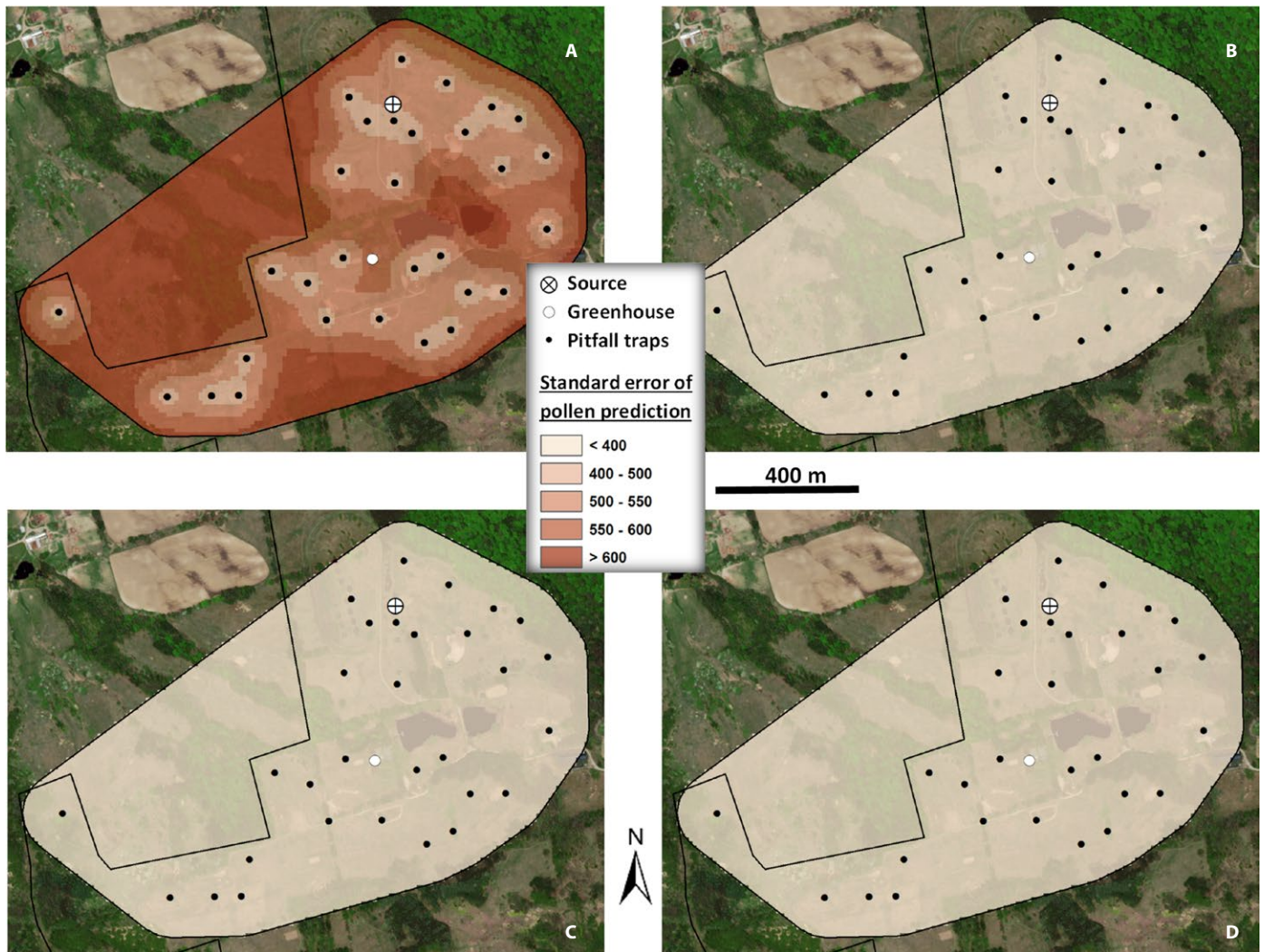


FIGURE 4. Prediction of standard error of pollen grains. Each map depicts the standard error of predicted pollen counts for treatments shown in Fig. 3 representing experimental treatments of artificial pollen grains applied to 100 individual flowers (A), 50 individual flowers (B), five individual flowers (C), and a control (D). Locations of source and potential pollen locations at the greenhouse are depicted using the same symbols as in Fig. 2.

in the vicinity of two sampling sites, and the source plant was located between them (Fig. 3A). As neighboring points are used to make predictions at unsampled locations based on a weighting scheme derived from the variogram, we see how pollen was predicted to be relatively high in areas that were unsampled (outside of the black boundary line, Fig. 3A) because neighboring points included samples located close to the source plant. These areas can be interpreted in concert with prediction errors (Fig. 4A). Prediction errors are highest in areas that were not sampled, and we are least confident in these values (darkest red zones on Fig. 4A). Prediction errors are lowest close to sampling sites; moreover, prediction errors are lower overall when the magnitude of semivariance in the response variable is lower overall.

Ordinary kriging was also used to interpolate the other experimental treatments, including the control treatment in which no paint pellets should have been available. However, there was very little spatial structure for treatment population sizes less than or equal to 50 labeled flowers. These results are depicted in the form

of continuous maps of predicted number of paint pellets (artificial pollen) across the sampled area (Fig. 3). Prediction errors provide an indication of where sampling density was lowest and where variability in the number of paint pellets detected was highest (Fig. 4).

We found the largest abundance of paint pellets in two pan traps close to our experimental source population for the treatment size of 100 individual labeled flowers (Fig. 3A). However, pan traps closest to the treatment source location did not necessarily have the highest number of artificial grains detected, and this may be because these samples were at lower elevations or they did not have showy flowers in their vicinity, which could attract more pollinators (as per Table 2 results). Adding covariates to the interpolation may improve predictive ability, but for this initial case study, and with a relatively small sample size of 30 samples, we selected ordinary kriging as a proof of concept to see if we could narrow in on the location of the source population.

The best results were obtained with the highest experimental treatment size (100 labeled flowers, Fig. 3A). Surprisingly, we were

TABLE 2. Statistical models predicting artificial pollen detection at the Koffler Scientific Reserve in King City, Ontario, Canada, based on *Brassica rapa* population size, proximity of showy flowers, elevation, and insects collected in a pan trap. Results of ANCOVA model predicting the abundance of artificial pollen at a sampling point (Poisson distribution, log link function, $n = 274$) for source population size, the presence of showy flowers in the local neighborhood of the pan trap, the elevation of sampling location, and the number of insects collected in pan trap.^a

Parameter	β (SE)	df	F	P
Population size	—	3267	3130.00	<0.0001*
Flowers in neighborhood	—	1267	361.98	<0.0001*
Elevation of sampling location	0.042 (0.0008)	1267	2833.74	<0.0001*
No. of insects in sample	-0.008 (0.0016)	1267	23.78	<0.0001*

Note: β = slope of the covariate; df = degrees of freedom; F = fixation index.
*Significant effects.

not able to fit a decent spatial model to samples taken during the experimental treatment of 50 labeled flowers; that is, spatial structure broke down very quickly for this treatment size, such that neighboring pan traps were not necessarily similar in the number of artificial pollen pellets detected. Despite this finding, all of the highest-density locations where paint pellets were detected were close to the source of the 50 labeled flowers treatment (data not shown due to color legend categories on Fig. 3B). Finally, as expected, the control population size with zero labeled flowers showed no detectable spatial structure in artificial pollen detected. In fact, artificial pollen pellets were detected under the control treatment, but only at one pan trap. In the single event where we found 10 artificial pollen pellets under population size of zero, the sample trap that collected the paint pellets was 240 m from the source population and 230 m from the greenhouse population. This seems to be a consequence of delayed pollen dispersal.

The abundance of artificial pollen collected differed significantly based on the size of the source population, where more artificial pollen pellets were collected when the source population was larger (Table 2, Fig. 5A). When showy flowers were common in the surrounding dominant vegetation, the abundance of artificial pollen pellets collected significantly increased by 39% relative to locations where the vegetation was grass-dominated (Table 2). Sampling sites at higher elevations tended to collect more artificial pollen pellets than sampling sites at lower elevations (Table 2). As the abundance of insects in pan traps increased, we captured significantly fewer artificial pollen pellets (although the difference was perhaps not biologically significant [$\beta = -0.008$]). Although the likelihood of detecting artificial pollen was higher when showy flowers were locally abundant (Table 2), this trend was not influenced by the number of insects caught in pan traps.

DISCUSSION

Artificial pollen tended to be transported by insects between patches of flowers offering food resources, and artificial pollen pellets tended to move short rather than long distances. Therefore, pollen dispersal can be exploited as a tool to detect plant populations within a landscape. This result is of applied interest not only because it creates a new tool for invasive species weed control but also because this tool could accommodate imperfect detection and high false

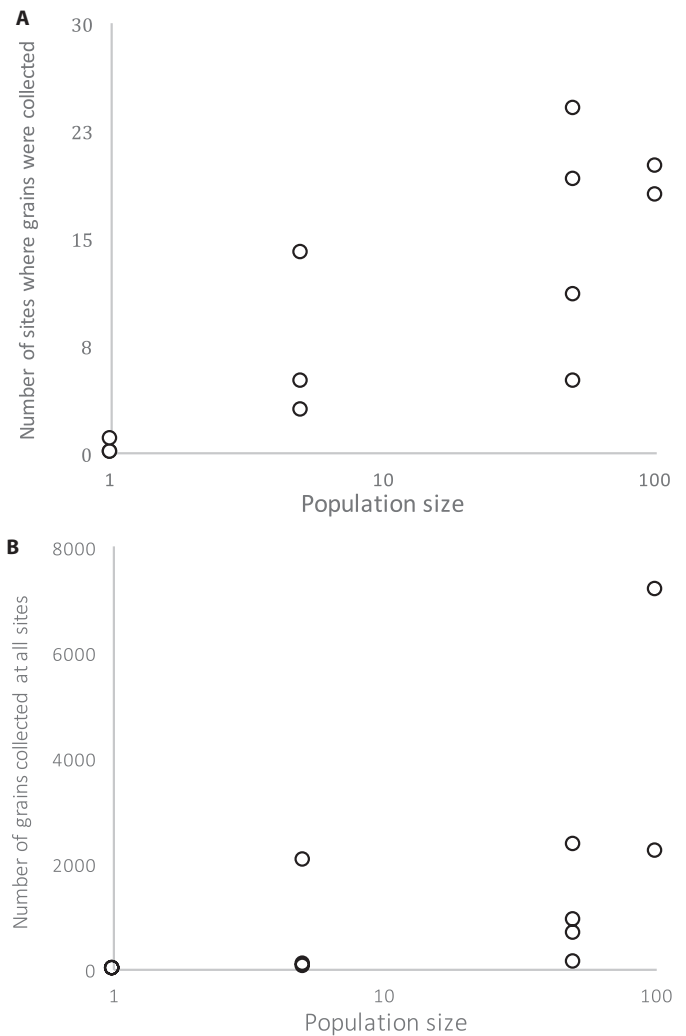


FIGURE 5. The effect of pollen source population size (graphed on a log scale) on the ability to detect populations based on collecting artificial pollen grains at 30 locations randomly located at the field site (Koffler Scientific Reserve, King City, Ontario, Canada) as determined by the number of sampling sites where artificial pollen grains were collected (A), and the number of artificial pollen grains found at all sampling sites (\pm SE) (B).

absence rates in a plant survey at the edge of an invading species' range or in endangered species surveys. Plant surveys at the edge of a range are often vulnerable to false absences because of reasonable limitations placed on search effort, especially those performed by line transects (Rew et al., 2006; Buckland et al., 2007). Our approach was able to hone in on a smaller area for searching, thus potentially reducing field search time. Future work will need to test this pollen sleuthing approach using various sampling regimes and a broader phylogenetic context to represent species with diverse life histories and a diversity of ecosystems.

Artificial pollen pellets, dispersed by wind vectors or insect vectors, are easy to capture over an old field landscape. The amount of artificial pollen captured in pan traps, dispersed from a single point source, was dramatically higher in large (100 individuals) versus very small populations (five individuals). Importantly, when populations were small, the rare plant could evade detection.

Consistent with results from other studies that experimentally manipulated population size and with published theory, as population size increases—not surprisingly—more pollen (artificial or otherwise) was dispersed out of populations (Slatkin, 1987; Klinger et al., 1992; Richards et al., 1999), a pattern likely influenced by the behavior of pollen vectors (Cresswell and Osborne, 2004). Although reduced population size reduces the volume of pollen produced by that patch, studies generally report that the amount of gene flow into and out of small proximate populations (i.e., even as small as two individual plants) is similar to that with large populations that are at moderate to long distances apart (Klinger et al., 1992; Richards et al., 1999); this finding is likely due to the fact that pollinators do not remain within small populations very long (Cresswell and Osborne, 2004). A second result that was surprising in this study was the slight reduction of pollen grains captured with increased numbers of insects trapped. We hypothesize that this decline may reflect either that insects were less likely to have the artificial pollen on their bodies or that it was more difficult to separate the artificial pollen from their bodies. However, if pollen censuses are systematically completed, this pollinator movement should serve to increase the likelihood that small populations will be detected using our pollen sleuthing technique, and therefore the proposed method is likely to have broad applications to other study systems.

Elevation also significantly influences source population detectability. For example, our pan trap sampling sites were most successful at collecting insects that carried artificial pollen when the pan traps were located at elevated points in the landscape. The complex effects of local topography on pollen dispersal are well documented (Markgraf, 1980; Fall, 1992; Van de Water et al., 2007; Pasquet et al., 2008). For example, although air movement is known to take insect vectors of pollen (and pollen itself) upslope and downslope, pollen is more likely to be found at hilltops rather than valleys (Markgraf, 1980; Fall, 1992). Moreover, constricted passes may increase the distance traveled by pollen (Van de Water et al., 2007). Thus, elevation may complicate efforts to model the location of small populations relying only on pollen density and may limit, in some cases, the implementation of a pollen sleuthing system.

Similarly, floral display is known to influence pollen movement across the landscape (Willson and Rathcke, 1974; Richards et al., 1999; Cresswell and Osborne, 2004). Pan-trap sampling sites were most successful at detecting insect-dispersed artificial pollen when these sites included showy flowers rather than grass-dominated microsites. Generally, floral display size increased pollen movement between patches (Richards et al., 1999), perhaps because it increased patch attractiveness for insect visitors, increasing geitonogamy, which may reduce pollen movement among plants within patches (Richards et al., 1999; Cresswell and Osborne, 2004). Furthermore, as floral display size increases, excess pollen will be produced, which should increase pollen movement out of patches (Willson and Rathcke, 1974). Therefore, species that tend to produce large floral displays and larger patches will be easier to detect using our sleuthing system than species that produce few flowers per plant or exist in very small patches.

There were two potentially important factors that could influence the applicability of the proposed technique but that were not considered in this paper. The first factor is the extent to which pollinator body size can influence pollen movement. Although pollinator body size does not influence the rate of pollen collection in brassicaceous plants (Sahli and Conner, 2007), the distance pollen moves by different insect vectors is largely correlated with their

foraging distance and body size (Nielsen and Ims, 2000; Ghazoul, 2005; Hensen and Oberprieler, 2005), which, in turn, may influence the uncertainty of estimates of pollen source locations. The second factor is the extent to which landscapes and population sizes of flowering plants influence the body size composition of the vector community (Sowig, 1989; Woodward et al., 2005; Greenleaf et al., 2007). When the vectors' communities differ in body size, this may influence the average distance pollen moves. Thus, future use of this tool would benefit from a stronger understanding of the dispersal distance of insect vectors and pollinator community of any particular focal plant.

Although we demonstrate the potential of pollen sleuthing as a detection tool for invading species, we are aware of limitations, such as upper and lower threshold plant densities, and/or minimum pollen inter-trap distances where this tool will be most useful for detecting invasion fronts. Dispersal varies with pollen abundance, and these differences were clearly evident in our model variograms (Fig. 3A–D, insets). For example, the range of detected artificial pollen got larger as pollen abundance increased (Fig. 3B compared with Fig. 3A), whereas at very low levels of artificial pollen (five painted grains and the control [Fig. 3C, D]) spatial structure was not evident. Therefore, a dispersal kernel would be undetectable at very low pollen levels. The ability to locate populations was improved in sampling sites with high pollen capture and low variance in pollen capture. Figure 4 depicts prediction error in modeled values of pollen abundance across the sampled area. The beauty of geostatistical methods is that once a variogram is known and a model of spatial structure for pollen dispersal is quantified, prediction error can be estimated anywhere in the study area (regardless of whether a given location was sampled or not). Prediction error can be used to direct sampling efforts (Melles et al., 2011) such that errors are minimized in the vicinity of locations with the highest detected pollen pellets. We expect that there is an upper threshold of plant density at which the tool becomes less useful than human surveys, but this high density would be more typical of a mature invasion rather than an invasion front. Because of the existence of these thresholds, the pollen sleuthing system could be used as sampling sentries scattered on landscapes for remotely tracking invasion fronts, or for evaluation of demographic consequences of eradication efforts. Furthermore, because detection success varied among days, it would be important to incorporate sampling on multiple days in a future study.

The natural next step to development of this pollen detection system will be use of DNA metabarcoding, or a similar genetic tool, able to identify pollen capture at the species level. Advancement of this technology into environmental DNA (eDNA) metabarcoding, which allows for identification of a single species within a multi-species environmental sample, has given scientists a useful tool for biodiversity estimates (Dejean et al., 2011; Knox et al., 2012; Bell et al., 2016a, 2016b, 2017a, 2017b) and has proven to be a useful method to identify species too cryptic to be identified by morphological methods alone (Hebert and Gregory, 2005; Kress, 2005; Hajibabaei et al., 2007; Janzen et al., 2009). For instance, when using species-specific DNA barcodes, DNA extracted from environmental samples (sediment sampling, soil cores, water samples) can reveal the identity and distributions of individual species (e.g., Hofreiter et al., 2003; Willerslev et al., 2003; Ficetola et al., 2008; Jerde et al., 2011). Methods for pollen DNA metabarcoding (a type of eDNA barcoding) were only recently developed to use DNA barcodes to identify individual species in mixed pollen samples from air or insect vectors (Folloni et al., 2012; Keller et al., 2015; Bell et al., 2016a, 2016b, 2017a, 2017b), but

may prove similarly useful for revealing species distribution, abundance, and other spatial information. The more specific advantage of e-barcoding is in its ability to capture a DNA signal from mobile individuals (i.e., extra-organismal DNA [Ficetola et al., 2008; Hajibabaei et al., 2012; Barnes and Turner, 2016]). Although plants themselves are stationary organisms, pollen is predictably mobile and thus possesses great potential for broad application of barcode-assisted pollen sleuthing surveys, especially for the detection of source populations of invasive or rare and endangered species.

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APPENDIX 1. Geographic coordinates for the locations of the pan traps used in the study.

Trap no.	Latitude	Longitude
Trap 1	-44.03388889°N	-79.53805556°W
Trap 2	-44.03333333°N	-79.53666667°W
Trap 3	-44.03305556°N	-79.53972222°W
Trap 4	-44.03277778°N	-79.53527778°W
Trap 5	-44.03250000°N	-79.53916667°W
Trap 6	-44.03250000°N	-79.53833333°W
Trap 7	-44.03250000°N	-79.53444444°W
Trap 8	-44.03222222°N	-79.53777778°W
Trap 9	-44.03222222°N	-79.53611111°W
Trap 10	-44.03166667°N	-79.53361111°W
Trap 11	-44.03138889°N	-79.54000000°W
Trap 12	-44.03138889°N	-79.53500000°W
Trap 13	-44.03111111°N	-79.53833333°W
Trap 14	-44.03000000°N	-79.53361111°W
Trap 15	-44.02944444°N	-79.54000000°W
Trap 16	-44.02944444°N	-79.53694444°W
Trap 17	-44.02916667°N	-79.54222222°W
Trap 18	-44.02916667°N	-79.53777778°W
Trap 19	-44.02888889°N	-79.54111111°W
Trap 20	-44.02861111°N	-79.53611111°W
Trap 21	-44.02861111°N	-79.53500000°W
Trap 22	-44.02833333°N	-79.54888889°W
Trap 23	-44.02805556°N	-79.54055556°W
Trap 24	-44.02805556°N	-79.53888889°W
Trap 25	-44.02777778°N	-79.53666667°W
Trap 26	-44.02750000°N	-79.53750000°W
Trap 27	-44.02722222°N	-79.54305556°W
Trap 28	-44.02638889°N	-79.54555556°W
Trap 29	-44.02638889°N	-79.54416667°W
Trap 30	-44.02638889°N	-79.54333333°W