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Online tool for GR horseweed (*Conyza canadensis*) gene flow

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Abstract. Horseweed (*Conyza canadensis*) with evolved glyphosate resistance (GR) has become an especially problematic weed in crop production across the United States. This paper reports on the framework development of an Internet decision tool that can be used by regulatory agencies, industries, farmers, and the public. It estimates the potential gene flow from GR horseweed and can offer guidelines for the prevention of gene flow under different meteorological and environmental conditions. The online tool is based on the Lagrangian dispersion model Hysplit4.0 which simulates local and regional dispersion, deposition, and outcrossing of horseweed pollen and seeds. The tool has user-friendly "Googlemap" interfaces.

Keywords. Horseweed, gene flow, simulation, model, Lagrangian, pollen, seed, dispersion, deposition

Introduction

Horseweed (*Conyza canadensis*) with evolved GR has become an especially problematic weed in crop production across the United States. This plant is considered an important agricultural weed because it can reduce agricultural yields by 90% at high densities and becomes problematic with low-tillage agriculture (Bruce and Kells, 1990; Shields et al., 2006). The spread of GR horseweed has been rapid, with resistant populations covering millions of acres in corn (*Zea mays* L.), soybean, and cotton (*Gossypium hirsutum* L.) fields in the U.S. (Heap, 2011).

Because of the importance of horseweed, several gene flow studies were conducted (e.g., Bruce and Kells, 1990; Dauer et al., 2006a, 2006b, 2007, and 2009; Henry et al., 2008; Regehr and Bazzaz, 1979; Shields et al., 2006; Weaver, 2001). Horseweed is self-compatible (Shields et al., 2006). Smisek (1998) found that on average, 96% of florets were self-pollinated, and the outcrossing rate was only about 4%. Therefore, horseweed gene flow studies have focused on seed spread (Dauer et al., 2006a, 2006b, and 2007; Shields et al., 2006) instead of pollen transport, pollination, and outcrossing. Even for seed spread, there remains an atmospheric zone (6–68 m, >120 m above ground level) within the surface layer that has been rarely explored (Dauer et al., 2009), and seed deposition on the ground was not measured. Modeling work also focused on seed spread using empirical and Gaussian models in Dauer et al. (2007 and 2009).

However, the transfer of GR via pollen as a mechanism of gene flow, in addition to long distance seed movement, is troubling because it could aid in the evolution of multiple modes of resistance (Henry et al., 2008), and even a single plant can produce more than 200,000 seeds (Bhowmik and Bekech, 1993; Weaver, 2001).

Horseweed develops elongated flower stalks (bolts) in late spring and blooms in mid-July, and produces seed from early August through September (Shields et al., 2006). Plants can reach 2 m in height, and a single plant can produce more than 200,000 seeds that are wind-borne with the aid of a pappus (Bhowmik and Bekech, 1993; Weaver, 2001). Horseweed seeds are lightweight, with a gravitational-settlement velocity of 0.323 m/s (Andersen, 1993; Dauer et al., 2006).

Although horseweed pollen and seed dispersal is described as wind-borne, there is little knowledge, preventive guidelines, and mechanism modeling on gene flow (dynamic pollen and seed release rate, dispersion, deposition, and final outcrossing). In particular, little information is available on the relationship of gene flow to atmospheric conditions (wind speed, direction, wind variability, and atmospheric stability), buffer plant type, height, and size, and GR horseweed patch size. This proposed project will provide this needed information.

Spatial and temporal extrapolation of gene flow measurements are most efficient using numerical models. The most useful models for gene flow will be those that can predict the pollen and seed release, transport, deposition, and final outcrossing from individual locations and then scale up to landscape and regional scales.

The objective of the project is to develop internet decision tools and provide data through modeling that can be used by regulatory agencies, industries, farmers, and the general public about the potential gene flow from GR horseweed and to offer guidelines for the prevention of gene flow under different meteorological and environmental conditions.

Methodology

The framework of an Internet tool was developed for horseweed gene flow (http://rsetserver.sws.uiuc.edu/horseweed/).

Figure 1 shows the general flowchart of this gene flow model for GR horseweed outcrossing. The gene flow model consists of four major components (submodels): pollen source strength, dispersion, deposition, and final outcrossing. By inputting species, field dimension, and weather data, the model predicts the dynamic three-dimensional pollen concentrations and two-dimensional depositions, and the final two-dimensional outcrossing ratios in the receptor plants.

Pollen source strength

The pollen source strength $Q_0(t)$ (grains/plant/s) is predicted according to time (t) and weather data (wind speed, solar radiation, humidity, precipitation) by using the empirical regression equation that will be obtained in future experiments (see the section of **Ongoing Experiments**) (The equation will be deduced using

regression analysis from the experimental data). In the current online tool framework, the source strength was set to 1 pollen grain/plant/s during a pollination season.

Pollen dispersion/deposition

The NOAA Hyslit4.0 (a Lagrangian model) is used to simulate dispersion and deposition. The Hysplit4.0 model simulates puff advection for different particle sizes (including pollen size and seed size) after Draxler and Hess (1997).

NAM12Km (12 km resolution, 2007-present, ftp://arlftp.arlhq.noaa.gov//pub/archives/nam12) and NARR 32Km (32 km resolution, 1997-present, nomads.ncdc.noaa.gov/data.php?name=access#narr_datasets) 3-hour meteorological data were downloaded and used for the Hysplit 4.0 as weather data inputs. The model can run for multiple years using the meteorological data, and then the average of the simulations are outputted.

Outcrossing

The outcrossing ratio (*OutR*) is predicted according to the grand total pollen deposition flux (*GTD*). The empirical regression relationship between *GTD* and *OutR* derived from the ongoing experimental data will be used for this purpose. The equation is as follows:

$$OutR = 3 \times 10^{-8} \times GTD$$

Seed dispersion

The seed dispersion from the source plants after a pollination season was simulated using Hysplit4.0 also.

Implementation of the online tool

The implementation of the online gene flow tool consists mainly of two parts: the user interface "driver.php", which the users directly interact with, and the background serving process "daemon.php". The diagram of this framework is shown in Figure 2.

The user interface "driver.php" allows new users to register, and a folder is created on the server corresponding to each user. The user information such as username, password, and e-mails are stored in a MySQL database. Every time a client tries to log in, their identity will be checked using the information stored in the database and thus the links to the computation results will be sent to users using the corresponding e-mail. After the user logs in, he/she can choose to perform a pollen/outcrossing dispersion simulation or seed dispersion simulation. The starting date and the period in terms of weeks or years can be input manually. The location of interest can be chosen using a Google map embedded in the user interface. All these parameters are stored in another MySQL table.

The background serving process is running a PHP script, which queries the table in the database, and if the table is empty, it will go to sleep; otherwise it will retrieve the parameters, perform the computation, and send the results to the user. The PHP script runs as a background process on Windows Server 2008 using Windows task scheduler. Rather than using a 3rd party e-mail server, a SMTP server is set up on a local machine in order to send e-mails more efficiently and flexibly. The computation is mainly performed by Hysplit4.0 and related software. Hysplit is called by a jar file which is used to set up the configuration file for Hysplit and output the averaged pollen/seed concentration, deposition, and outcrossing at specific locations in terms of longitude and latitude. Jar files are also created to generate kml files containing contours of these parameters in the specified area. Finally, the kml file for concentration, deposition, and outcrossing is packaged with the legend into a kmz file. To make the contours accessible to machines not having Google Earth installed, Matlab script was written to generate contours in pdf and png formats using the built in function "contourf". To make it look like Google Earth, the "plot_google_earth" function is used to overlay the contour over the hybrid Google map (Mathworks, 2013). This script is compiled into an "exe" file using the Matlab deployment tool; thus it can be called by the PHP script. Finally, the links to all the generated files are sent to the users by e-mail for downloading.

Results and Discussions

The framework of the online tool was developed. Figure 3 shows the input interface. A user can click on the Google map or key in the latitude and longitude. Then the tool can calculate the pollen concentration, deposition, and outcrossing, and seed dispersion from horseweed plants during a pollination season. A user can choose many years of meteorological data as inputs to ensure that the average outputs are more statistically significant.

Figures 4-6 show the sample deposition, outcrossing ratio, and seed dispersion simulations during a pollination season. The source was from Nashville, TN. The pollen source strength was set to 4 pollen grains/plant/s during a pollination season (10,000 plants at the source). As expected, in the prevailing wind direction (Northeast), more dispersion occurred than in the upwind direction.

The tool also serves other functions. A user can choose multiple locations of interest to depict different sizes of GR horseweed patches on the Google map. The tool can then show the local and regional gene flow data on the Google map and output the data to a text data file. Users can also define their own weather data (e.g., stronger wind speed) and surrounding buffer plants by clicking on scroll-down lists. The tool covers the whole USA.

Future Experiments

To calibrate and validate the tool, field experiments will be conducted at the Plant Science Farm in Knoxville, TN (Figure 7). In the experiments, glyphosate susceptible (GS) horseweed will be used as receptor plants and glyphosate resistant (GR) horseweed will be used as source plants. One circular area (12 m diameter) of source plants will be grown, surrounded by the receptor plants (80 m zone in radial direction). The horseweed will be about 2 m high.

Pollen concentration sampling

In pollen dispersal experiments, pollen concentration will be measured using columns of Rotorod samplers with retracting-type sampling heads (Model 20, Sampling Technologies, Inc., MN, USA) in the sampling lines. One-column Rotorod samplers will be located in the center of the source to measure the horizontal flux (grains/m^2/s) profiles of source production and release. One additional sampler at flower height will be located at the center of the source to continuously record reference values of the source strength (pollen release rate) when the other samplers are switched off for rod replacement for the next sampling period. Other columns of Rotorod samplers will be set up in the prevailing wind direction. There will be three other sampling lines. In addition, two balloons will be used to hang Rotorod samplers (4 samplers/balloon) to sample pollen concentration at the height of 10 to 200 m in the downwind direction 100 m to 5000 m from the source. The pollen sampling height, the downwind distances, and the sampling time will be adjusted based on if it is detectable.

Pollen deposition sampling

Microscope slides (2.5 cm × 7.5 cm) covered with silicon grease (Sampling Technologies, Inc., MN, USA) will be used to measure the deposition at flower heights along the sampling lines.

Pollen concentration and deposition sampling will be conducted throughout each pollination season. The sampling period for the collectors will be 1 to 3 hr during the daytime (7:00-19:30). Timers on the Rotorod samplers will be used to provide different intermittent samplings, as determined by the capacity of the Rotorod samplers to avoid being overloaded. New fresh sampling rods and slides will be placed for each new sampling period.

Outcrossing

Seeds will be sampled to determine the rate of fertilization or seed production. At the location of each slide pole in the receptor field, four surrounding plants will be sampled.

Seed dispersal sampling

In seed dispersal experiments, the seed dispersal (release rate, concentration, and deposition) will be measured for the pollen dispersal measurements. But the receptor plants will be removed before the sampling so that the sampled seeds will be only from the source plants. Pollen sampling poles, balloon, and samplers will have the same setup as the pollen sampling except for the seed deposition which will be measured with the slide at the ground level. The sampling period will be 1 to 3 hours or longer, depending on if it is detectable.

Micrometeorological measurements

An automated weather station (Vantage Pro2 Plus, Davis Instruments, Hayward, CA) will be employed to measure a 30-min average of the meteorological parameters at the experimental sites, including solar radiation, precipitation, temperature, and relative humidity.

Virtual air temperature (T) and the stream wise (u), cross-stream (v) and vertical (w) wind components will be measured at 20 Hz sample rates with three Campbell Scientific Inc. CSAT3 three-axis sonic anemometers mounted at the height of above the crop canopies during the measurement periods in the source and receptor fields and at the first balloon location. From these data, 30-minute average statistics rotated into the mean wind stream will be calculated, after Kaimal and Finnigan, (1994). The averages are: wind direction (U_{dir}), mean wind speed ($\overline{u}$), virtual air temperature (Tv), the friction velocity(u*), and the stability parameter (Obukov length, L). The wind data will be used as inputs to the model.

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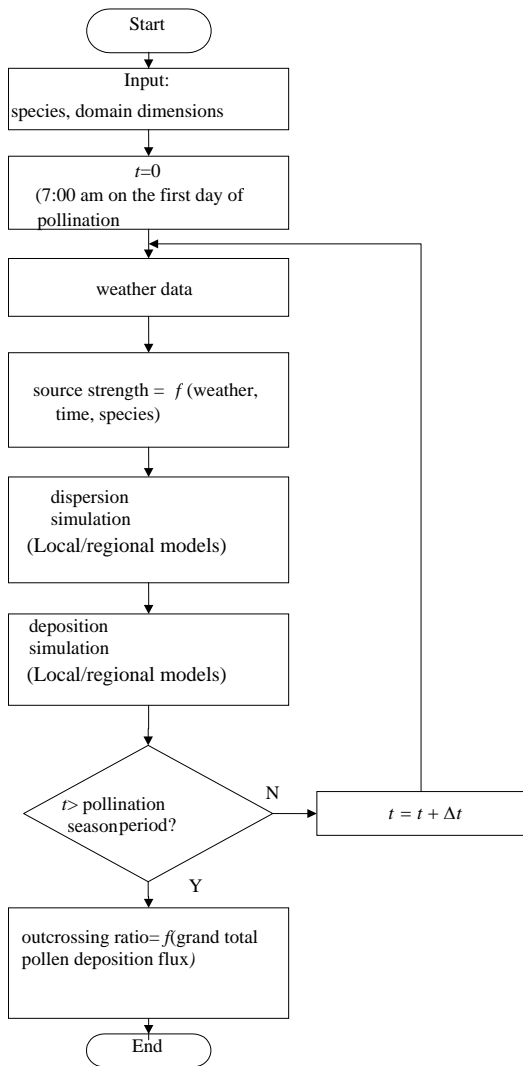Weaver, S. E., 2001. The biology of Canadian weeds 115. Conyza canadensis. Can. J. Plant Sci. 81: 867–875.

Figure 1. General flowchart of pollen release, dispersion, deposition, and outcrossing from GR horseweed.
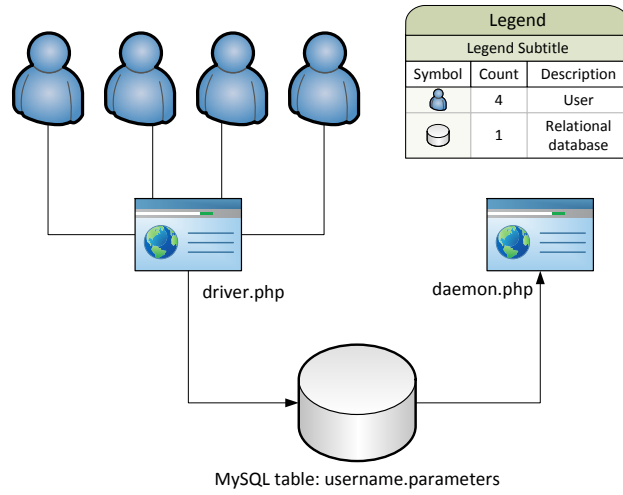
Figure 2. The general structure of the online tool implementation.
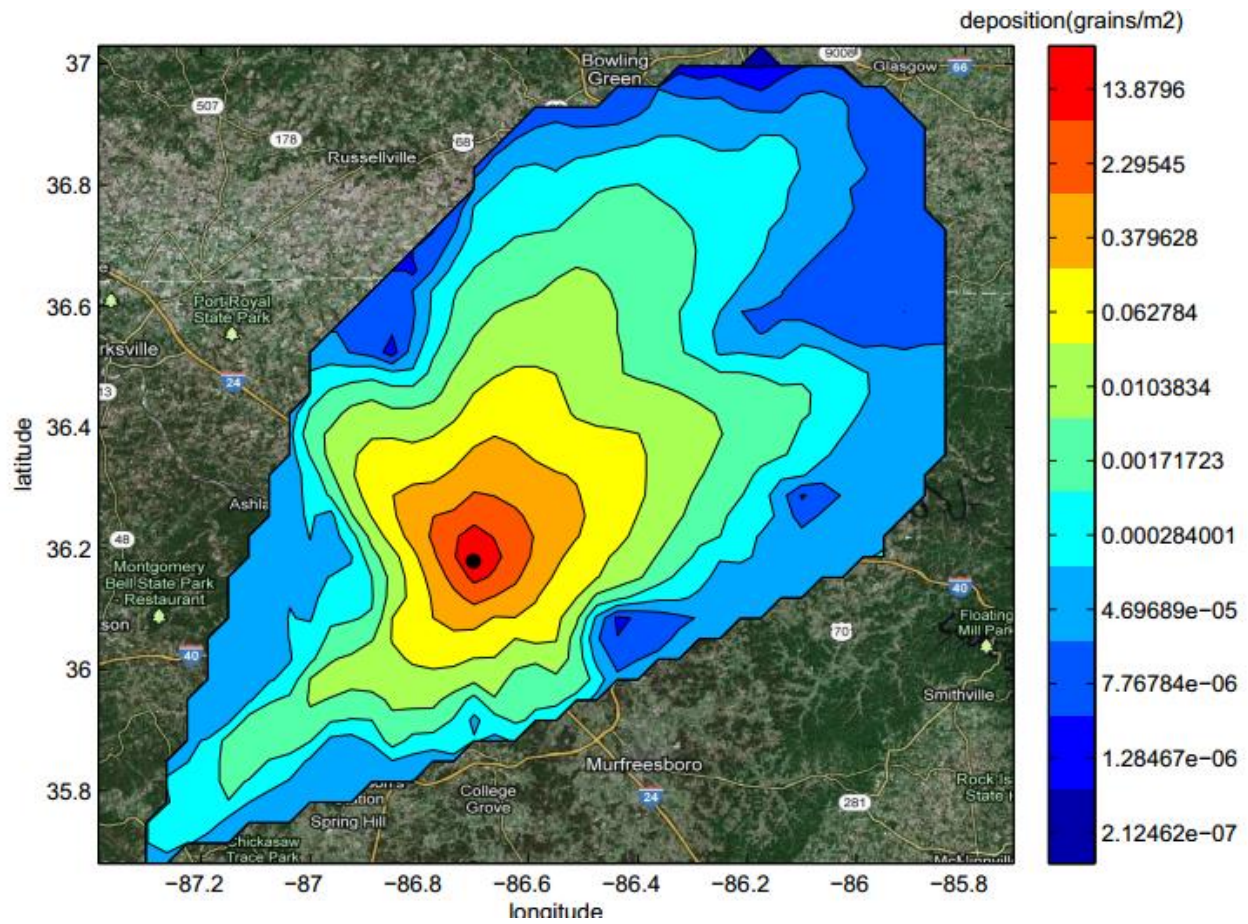

Figure 3. The input interface of the online tool.

Figure 4. The sample total deposition output of the online tool from horseweed plants during a pollination season. In the current online tool framework, the seed source strength was set to 1 pollen grain/plant/s during a pollination season (10,000 plants).
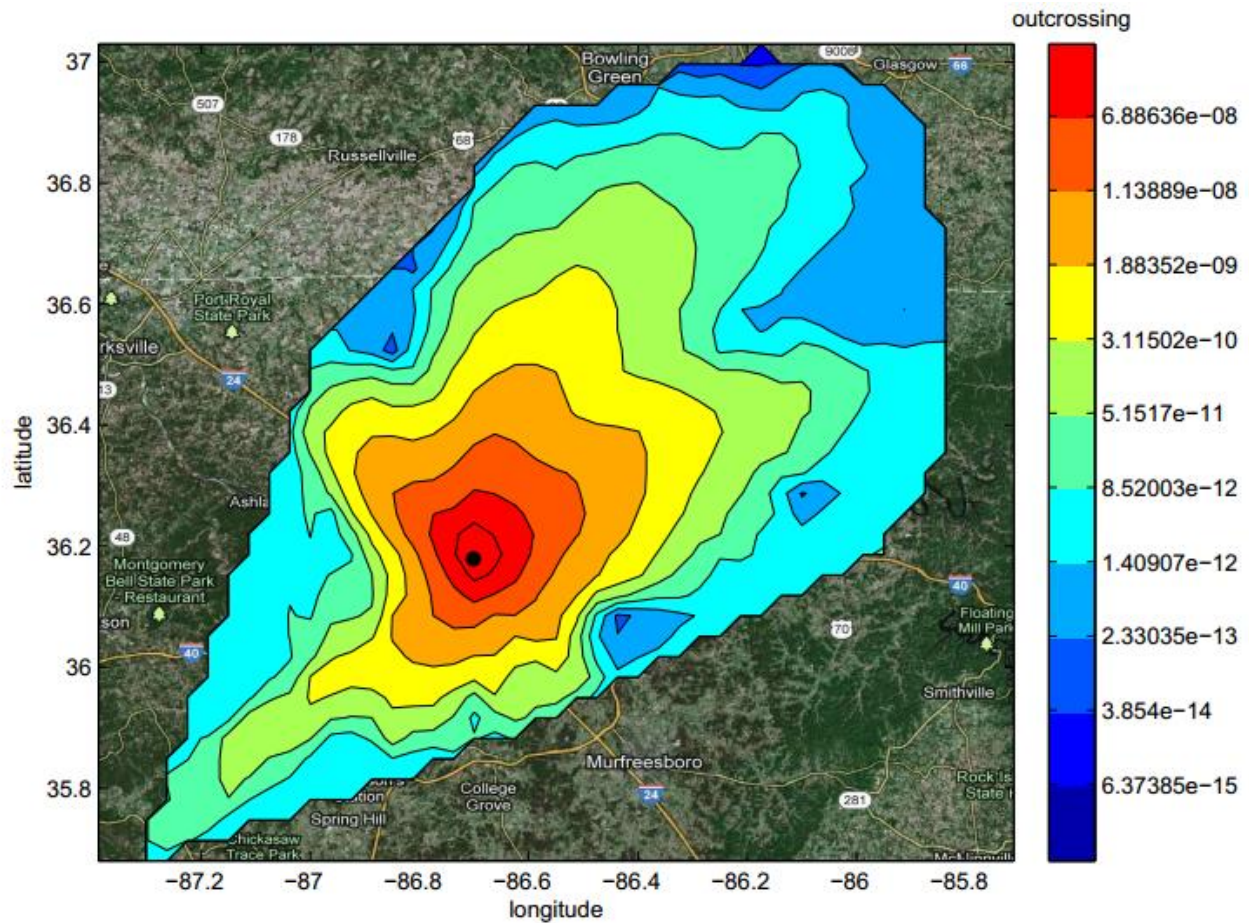
Figure 5. The sample outcrossing ratio output of the online tool from horseweed plants during a pollination season. In the current online tool framework, the seed source strength was set to 1 pollen grain/plant/s during a pollination season (10,000 plants).
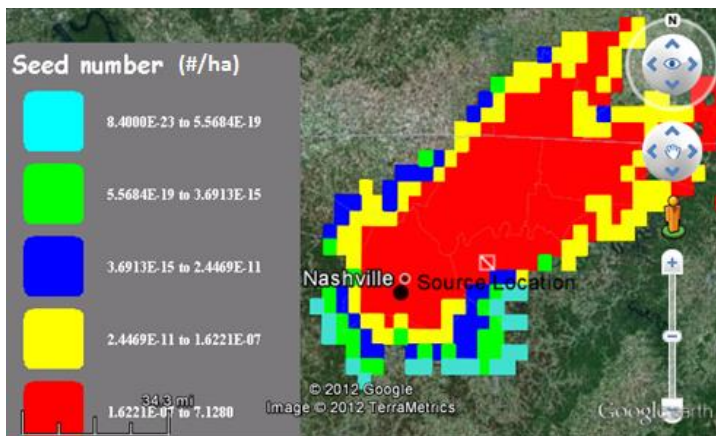

Figure 6. The sample seed dispersion output of the online tool from horseweed plants during a pollination season. The example shows the potential deposited seed number dispersed into the atmosphere from horseweed plants. In the current online tool framework, the seed source strength was set to 1 seed/plant/s during a pollination season (10,000 plants).

Prevailing wind

100-5000 m depends on if it is detectable

20 m

20 m

80 m   10 m 10 m   20 m

20 m

1 m

5 m

Receptor plants

Source
plants

Automated
weather station

40-120 m

20 m

10 m 10 m

10 m

2 m

2 m

2 m

2 m

2 m

1 m

2 m

1 m 1 m 2 m

1 m

1 m

1 m

1 m

Rotorod
(80-200 m)

○ Rotorod
(10 m)

○ Rotorod
(6 m)

◇ Rotorod
(short)
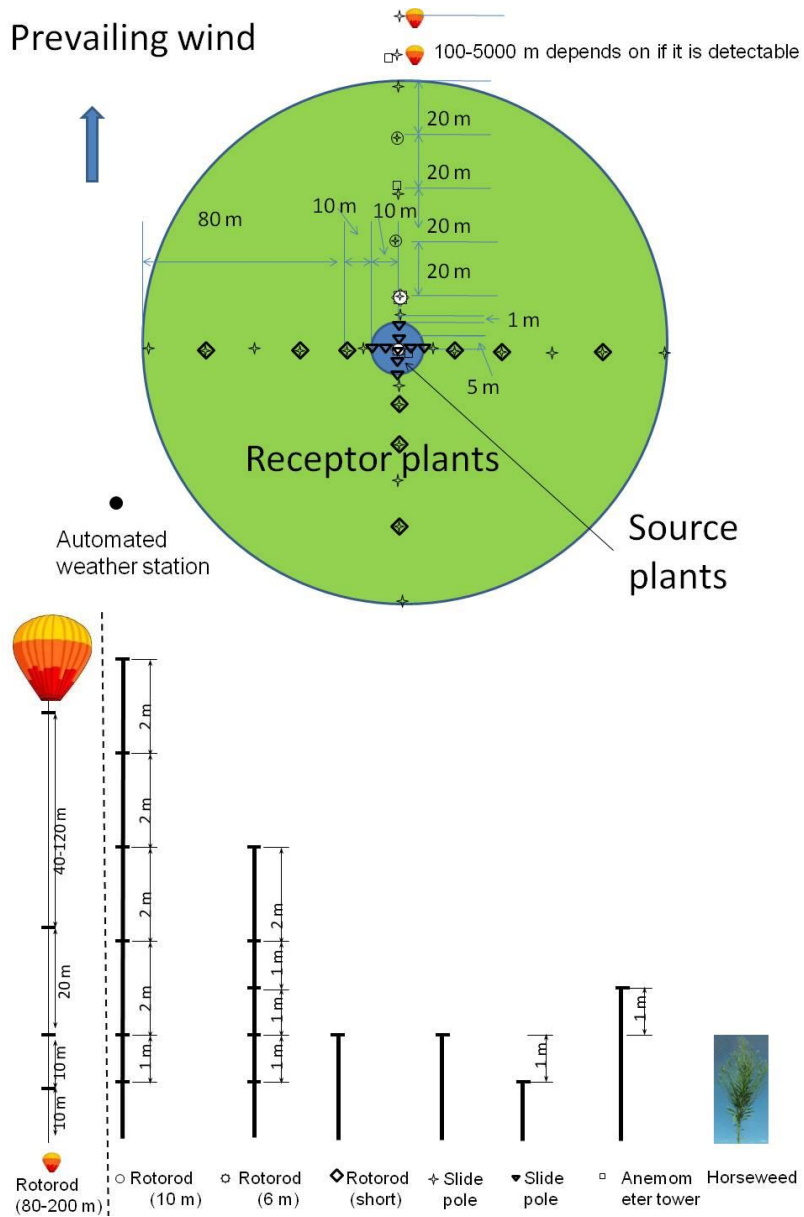
✦ Slide
pole

▼ Slide
pole

▫ Anemom
eter tower

Horseweed

Figure 7. The schematic sketch of the gene flow experiments.