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LONG-DISTANCE GM POLLEN MOVEMENT OF CREEPING BENTGRASS USING MODELED WIND TRAJECTORY ANALYSIS

Peter K. Van de Water,[1,3] Lidia S. Watrud,[1] E. Henry Lee,[1] Connie Burdick,[1] and George A. King[2]

[1]*National Health and Environmental Effects Research Laboratory, Western Ecology Division, U.S. Environmental Protection Agency Office of Research and Development, 200 Southwest 35th Street, Corvallis, Oregon 97333 USA*
[2]*Dynamac Corporation, 200 Southwest 35th Street, Corvallis, Oregon 97333 USA*

Abstract. The importance of understanding the role of atmospheric conditions in pollen dispersal has grown in recent years with increased field-testing of genetically modified (GM) crop plants. An atmospheric model was used to characterize wind trajectories at 10 m and 100 m above GM pollen source fields located within a 4452-ha "control" area north of Madras, Oregon, USA, designated by the Oregon Department of Agriculture (ODA). The area was used in 2003 for the growth of GM creeping bentgrass (*Agrostis stolonifera*) engineered to be resistant to glyphosate herbicide. The presence of the GM gene (*CP4 EPSPS*) provided a distinct selectable marker for pollen-mediated gene flow to sentinel and resident *Agrostis* spp. plants. Linkage of GM gene presence with wind flow characteristics over the "control" area became essential to understand the timing and processes leading to long-distance transport of this pollen. Wind trajectories showed a general pattern of northwest to southeast air movement. Trajectory travel distances calculated hourly from 06:00 hours to 15:00 hours during the 2003 pollination period (15 June–15 July) showed movement up to 15 km from the "control" area's center by the first hour. Maximum travel distances increased to 40 and 55 km after two and three hours from release, respectively. Calculated wind trajectory positions corresponded with observed long-distance pollen-mediated gene flow in the seedlings of sentinel and resident plants. The highest correlations were found during the late morning hours. Back-calculated wind trajectories from sentinel and resident locations with GM-gene-positive progeny suggested that most successful fertilizations occurred in the direction of prevailing winds during late June 2003. The occurrence of positive progeny from sentinel plants, upwind of the "control" area during this period, indicated the additional influence of local topography on pollen dispersal.

Key words: Agrostis gigantea; Agrostis stolonifera; *gene flow; HYSPLIT model; long-distance transport; modeled wind trajectories; pollen dispersal; pollen delivery.*

Introduction

Approximately 162 ha of genetically modified (GM) creeping bentgrass (*Agrostis stolonifera* L.) was planted for field testing in a "control" area as defined by the Oregon Department of Agriculture (ODA) north of Madras in Jefferson County, Oregon during August of 2002 (description *available online*).[4] The bentgrass was engineered to be resistant to glyphosate herbicide (RoundUp; Monsanto, St. Louis, Missouri, USA). *Agrostis* is one of the first perennial, wind-pollinated, and highly outcrossing transgenic crop plants tested for commercial use (Watrud et al. 2004). GM bentgrass is a cool-season grass, specifically developed for the golf-course industry in the northern tier states of the United States; an area where naturalized and native *Agrostis* spp. may also be found. The genus *Agrostis* is represented by >200 species worldwide, of which 26 occur within the United States and 14 are native to the state of Oregon (species list *available online*).[5] *A. stolonifera*, a cosmopolitan species, is known to hybridize with other *Agrostis* spp. as well as closely related members of the genus *Polypogon* (Wipff and Fricker 2001, Belanger et al. 2003). The widespread distribution of *Agrostis* spp. and *Polypogon* spp. suggests that gene flow between GM plants and compatible naturalized or native species can potentially occur in diverse geographic locations.

The GM bentgrass fields were planted in the ODA control area during 2002. In 2003, the first year of bentgrass flowering, fields were monitored for viable pollen dispersal in all directions, but primarily downwind from anticipated prevailing north/northwest winds at the time of anthesis. The GM gene for glyphosate resistance (*CP4 EPSPS*) was detected in the progeny of 75 plants at 73 locations where 138 sentinel *A. stolonifera* plants were deployed for six weeks. In addition, seeds from 16 of 30 *A. stolonifera* and 13 of

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[3] E-mail: P.VandeWater@comcast.net

[4] ⟨http://arcweb.sos.state.or.us/rules/OARS_600/OAR_603/603_052.html⟩

[5] ⟨http://plants.usda.gov⟩

39 *A. gigantea* resident plant locations showed evidence of pollen-mediated GM gene transfer. Based on extensive greenhouse and laboratory testing, glyphosate resistant (*CP4 EPSPS*) positive seedling progeny of sentinel and resident *A. stolonifera* and resident *A. gigantea* plants were found up to 21 km, 8 km, and 14 km from the control area boundary, respectively (Watrud et al. 2004). The greatest frequency of gene flow occurred within 2 km beyond the control area boundary.

Pollen of *A. stolonifera* is small (20–25 μm in diameter) and possesses an aerodynamic spheroid shape that facilitates atmospheric entrainment and travel. Once entrained in the atmosphere, wind speed, direction, elevation, and turbulence influence the distance and direction of travel from the initial release point. Under favorable atmospheric conditions the travel distance of a pollen grain can be extensive, e.g., on the order of hundreds to thousands of kilometers over many days (Wynn-Williams 1991, Bourgeois 2000, Van de Water et al. 2003). However, for successful GM bentgrass pollen-mediated gene flow, the travel distance from GM donors to compatible recipients must occur within the timeframe of pollen viability. Based on laboratory studies, the estimated time of *Agrostis stolonifera* pollen viability is approximately three hours (Fei and Nelson 2003). Within this three hour period dispersal distances can be considerable, given that between 15 June and 15 July 2003, daily (06:00–18:00 hours) wind speeds in Madras, Oregon, ranged from 1 km/h to 25 km/h (data *available online*).[6] Therefore, the maximum theoretical potential distance of the downwind spread of live pollen is 3–75 km.

This study characterized atmospheric conditions over the ODA control area during the 2003 pollination season (15 June–15 July). Wind characteristics (direction and speed) were analyzed for correspondence with the geospatial positions of sentinel and resident plants outside the ODA control area that showed evidence of pollen-mediated gene flow. It is well established that wind movement is the primary mechanism to disperse grass pollen. We tried to identify a relatively short period coincident with environmental conditions resulting in significant pollen release that met conditions for both pollen viability and anthesis. We analyzed daily wind characteristics within the 30 day period of potential pollination. We hypothesized that the geospatial distribution of GM-gene-positive seedlings from sentinel and resident plants should correspond with winds sweeping over the ODA control area prior to arrival at the positive sites. In addition, sentinel and resident plant progeny resistant to glyphosate were located upwind of, and at great distances along the mean wind direction, and would provide the most sensitive measure of the timing and duration of conditions affecting pollen dispersion, and downwind fertilization.

Methodology

The Oregon Department of Agriculture's (ODA) control area north of Madras, Oregon was designated during 2002 for the growth of genetically modified bentgrass (*Agrostis stolonifera*). The control area lies on the Agency Plains to the east of the Cascade Mountains, north of Madras, Oregon, on a basalt plateau with an elevation of 720 m (Fig. 1). The Deschutes River Canyon forms the northwestern boundary of the control area; it has feeder canyons to the southwest (Willow Creek) and along the north (Sagebrush Creek) to northeast (Mud Springs Creek) boundary. To the south and east of the control area, alluvial plains grade towards more distant mountainous regions. The climate of the area is dominated by the northern Pacific jet stream position. Cyclonic storms, generated in the north Pacific Aleutian low-pressure zone, provide instability across the region and are the main source of precipitation. The jet stream is generally more active during the fall, winter, and spring months. The control area lies just east of the Cascade Mountains resulting in a rain shadow effect and a buffer from the direct effects of these storm events. During the summer, high pressure builds from the southern deserts creating hot and dry conditions with an increased chance of late afternoon and evening thunderstorms (Ferguson 1999). Weather data for Madras (Coop #355142) is recorded at the local airport (40°40′ N, 121°9′ W; elevation 747 m) just outside the control area (see footnote 6). Weather information included maximum temperatures, wind speed and direction, solar radiation, and diffuse solar radiation for the period 15 June–15 July 2003.

Fields of GM *A. stolonifera* (bentgrass) were planted during the late summer of 2002 and flowered for the first time in 2003. Locations of the GM fields within the control area included five fields in the northwest corner, two fields in the southern portion, and one near the control area center (Fig. 1). Viable pollen movement was monitored during the summer of 2003 outside of the control area boundary by deploying sentinel plants of non-GM *A. stolonifera* along a designed multidirectional sampling grid extending 21 km to the south of the control area border (Watrud et al. 2004). Field observations for each location were used to further identify the period of potential fertilization. This included the date that sentinel plants were deployed and the timing of field watering during their period of exposure. The summer climate of eastern Oregon required periodic watering to ensure plant survival and fertilization potential. After pollination and panicle (seed head) formation, a period of approximately six weeks, sentinel plants were collected. Panicles of resident *A. stolonifera, A. gigantea*, and *Polypogon monspeliensis* plants were also collected in the surrounding area to test seedling progeny for potential hybridization between GM pollen and wild plant

[6] ⟨www.ocs.oregonstate.edu⟩

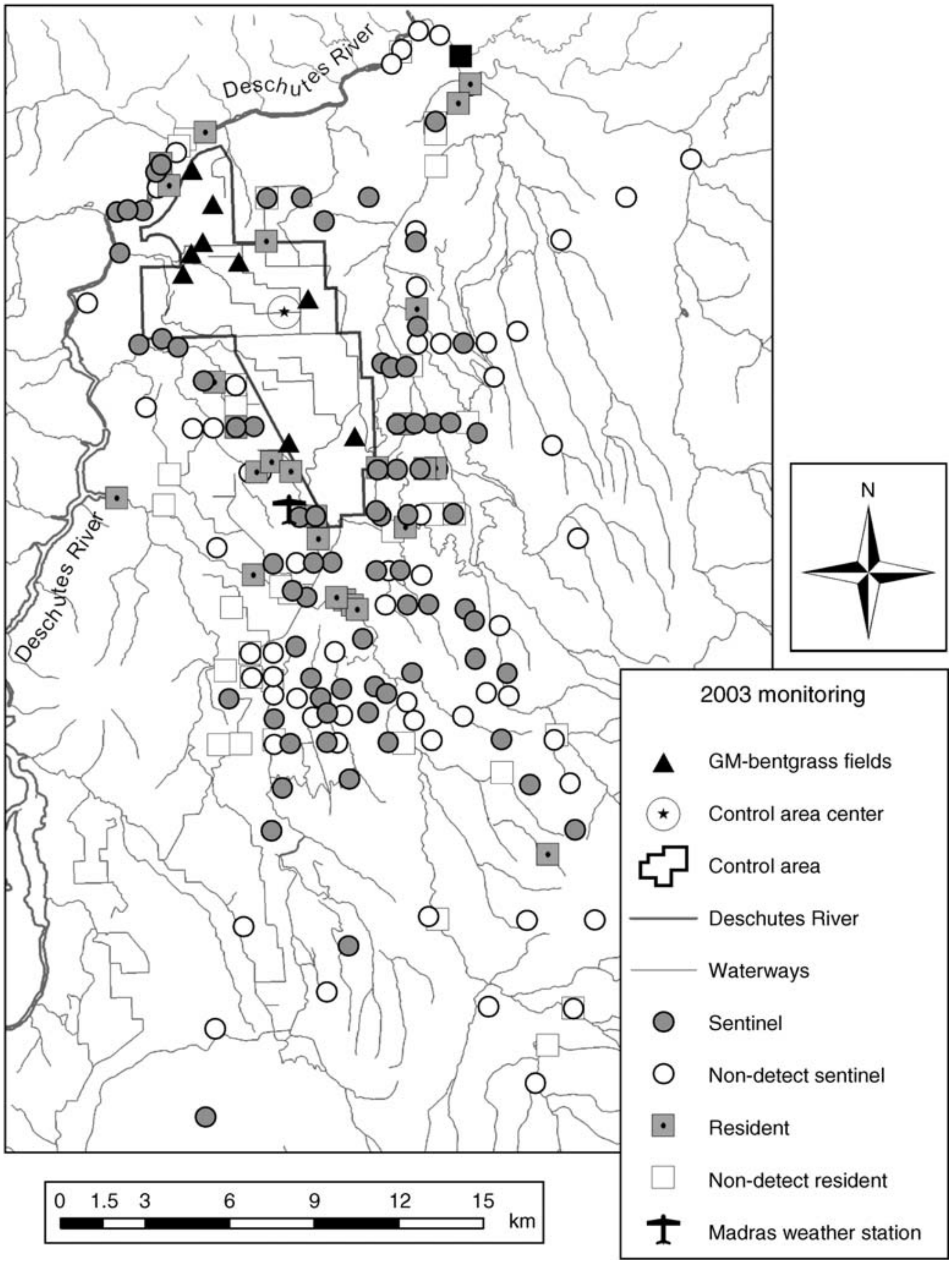

FIG. 1. Map of the Oregon Department of Agriculture control area showing the sentinel and resident plant locations. The single resident plant not crossed by wind trajectories arriving from the control area occurred to the northeast and is marked by a black square. Estimated positions of the individual fields are plotted along with the central point used to estimate the mean wind direction. The Deschutes River flows northward toward the Columbia River, bordering the northwest corner of the control area. Sentinel plant (circles) and resident plant (squares) locations producing GM-gene-positive, herbicide-resistant progeny are shaded; those producing progeny negative for the GM gene are not. Madras weather data were collected at the airport, southwest of the control district.

populations. Seeds from the sentinel and resident plants were tested for presence of the GM gene using multiple greenhouse and laboratory assays for herbicide resistance and for the presence of the GM gene, and its protein product (Watrud et al. 2004).

In 2003, the pollination period of *A. stolonifera* occurred from approximately 15 June to 15 July. Air mass trajectories originating from the control district center were calculated for this time period using the HYSPLIT-4 (hybrid single-particle lagrangian integrated trajectory) atmospheric model of single particle dispersion (Draxler and Hess 1998, Draxler 1999). The HYSPLIT-4 model estimates the location (latitude, longitude, and elevation) as a particle travels away from a fixed-height release point. The model uses a single particle trajectory calculated with mean wind components. Throughout, EDAS (National Weather Service's Eta Data Assimilation System) output was used to model the atmosphere with a 3-h temporal and 80-km spatial resolution. Vertical resolution may be as great as 250 km. Interpolation to finer scales in both space and time is linear. Modern measurements from the Madras weather station were used to show correspondence with modeled atmospheric conditions. Backward and forward trajectory analysis was used to examine the association between wind patterns and the spatial distribution of positive sentinel and resident plants. To characterize average wind movement across the control area, models were forward calculated from the control area's center at 10 m and 100 m heights (44°44′ N, 121°9′ W). Different modeled elevations were used to judge consistency between near surface winds (10 m) and more regional atmospheric wind patterns (100 m). Similarly measured wind direction at Madras, Oregon, was used to show consistency with modeled values. Hourly trajectory positions were computed with respect to GMT for the hours from 06:00 to 15:00 PDT, a period spanning estimates of pollen viability (Fei and Nelson 2003). Sunrise during this period ranged from 05:20 to 05:30 hours.

Calculated hourly air-mass positions, (latitude and longitude) were plotted as polar coordinates to characterize wind direction and wind run for the following three hours away from the starting point located in the control area's center. Descriptive statistics were calculated for the mean and standard deviation of wind direction at 10 m and 100 m starting height. The downwind air-mass trajectory position at the 10 m start height, measured from 0° at hours 1, 2, and 3, were calculated and grouped into 22.5° bins surrounding the control area center for each daily hour (06:00–15:00). The positions of sentinel and resident plants were treated similarly to compare against the position of the hourly air mass trajectories occurring during the 31-day pollination period. The percentage of downwind trajectories falling into each bin during hours 1, 2, and 3 were calculated, as were the sentinel and resident plant locations (Watrud et al. 2004). Statistical analysis used top-down linear correlation (Iman and Conover 1987), comparing weighted, ordered values for the top four occurrences of hourly endpoint trajectory positions against the weighted, ordered values in the same bins for the positive sentinel plant positions. Correlation coefficients were computed for each hourly occurrence.

To further delineate the timing of downwind fertilization, trajectories were back- calculated from 73 sentinel and 29 resident plant locations, respectively, where GM-gene-positive seedling progeny were found after fertilization in 2003. The atmospheric position was back calculated for the previous three hours (upwind) between 09:00 and 18:00 hours for each day from 15 June to 15 July. Thus, the travel of wind trajectories towards each location was calculated as early as 06:00 hours (three hours prior to the 09:00 hours trajectory) to just prior to the last calculation at 18:00 hours. The number of daily hourly trajectories crossing the control area prior to arrival at locations where GM pollen fertilization occurred was plotted against the distance of each location from the control area boundary. To further distinguish pollination conditions, days when trajectories tracked over the control area were plotted for both sentinel and resident locations. In addition, air-mass speed was calculated from the trajectory data for the period of interest in late June (22–27 June). Hourly trajectory positions determined the upwind straight line distance from each sentinel or resident plant location, and were used to estimate wind speeds.

Results

Madras, Oregon, weather station (Coop # 355142) wind direction data (see footnote 6) were used to determine the correspondence between measured and modeled wind parameters (Fig. 2). Mean hourly wind direction data (06:00–15:00 hours) showed increasing correspondence, measured as the degree difference between modeled and measured wind direction, during each day. A significant shift in variability occurred between 09:00 and 10:00 hours, with most values showing less than a 90° difference in the afternoon hours, but with greater variability earlier. This shift is consistent with increased wind speed from morning to afternoon (data not shown). Modeled winds were consistently from the north to northwest after 10:00 hours with most measured winds blowing clockwise of the modeled wind position (Fig. 2). Prior to 10:00 hours, wind speeds were light and variable, between 0 and 6.4 km/h, with a majority of measured wind directions counterclockwise of the modeled trajectories.

A total of 310 first-hour modeled wind trajectory positions calculated from the control area show a predominant wind direction from the northwest toward the southeast (hour 1 mean wind direction [±SD], 10 m, 304° ± 50°; 100 m, 317° ± 65°). With the hour 2 and 3 trajectories added (a total of 930 hourly trajectory endpoints) the mean wind direction shifts slightly at 10 m toward a more northerly position but maintains its direction at 100 m (mean wind direction, 10 m, 310° ± 55° ; 100 m, 318° ± 63°; Table 1). Analysis of hourly wind speeds combined for the 31-day pollination period shows an overall increase from 06:00 to 15:00 hours (Table 2). The increase in afternoon wind speeds and greater correlation between measured and modeled wind direction resulted from increased surface heating and vertical mixing with faster moving winds aloft. The positions of sentinel and resident *A. stolonifera* and *A. gigantea* plants producing GM-gene-positive seedlings

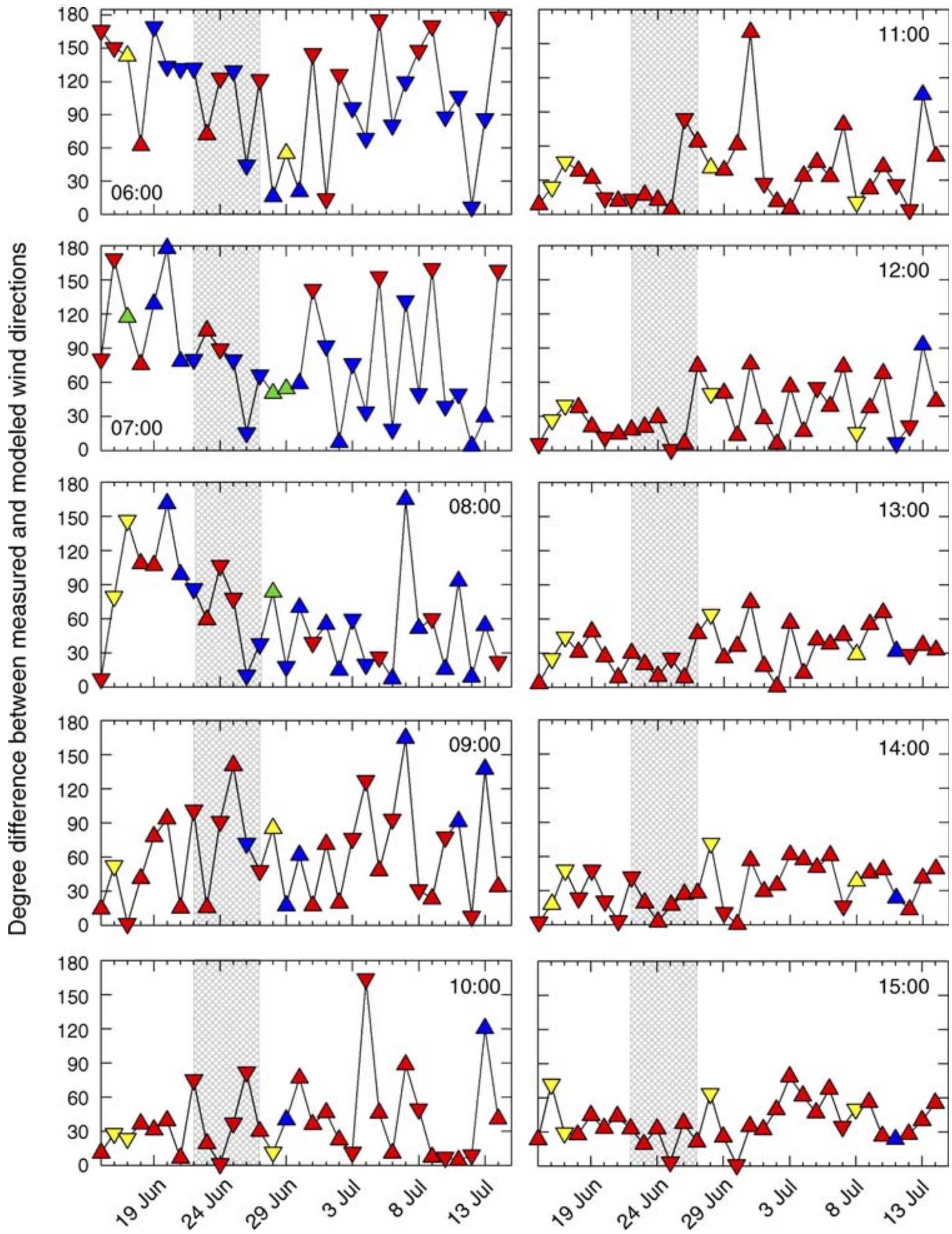

FIG. 2. Degree difference between hourly measured and modeled wind directions for 15 June–15 July 2003. Those points with an upward-pointing triangle are shifted clockwise from modeled trajectory directions, whereas those with a downward-pointing triangle are shifted counterclockwise. The triangles are color coded by the quadrant of the modeled wind direction (0–90°, yellow; 90–180°, green; 180–270°, blue; 270–360°, red). The shaded area defines the period of interest for potential pollination, 22–27 June 2003.

show a similar pattern (Fig. 1). That is, the majority of wind trajectory positions at 10 m (~70%) and plant positions resulting in GM gene transfer to their progeny (67% sentinel and 44% resident *A. stolonifera* along with 69% resident *A. gigantea* plant locations) occurred in the downwind direction, i.e., the southeast quadrant comprised of areas 5 to 8 (Tables 3 and 4). Of the remaining locations resulting in GM-positive seeds, the highest percentage of sentinel plants occurred in area 9 (11%), the easternmost region of the third quadrant. Remaining resident populations with GM-positive progeny were more scattered and the small number of representatives failed to show a distinct pattern.

Pollen source concentrations are variable, i.e., the source supply varies over the pollination season and changes with daily environmental conditions during the period of anthesis. Pollen release in wind-pollinated plants follows a normal distribution with an elongate

TABLE 1. Percentage of the total wind trajectories falling within 22.5° (0.3927-radian) slices (1–16) of the Oregon Department of Agriculture's control district (44°44′ N, 121°9′ W).

	Time of day									
Theta defined areas	06:00	07:00	08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00
Hour 1										
0.0–22.5° (1)	6.5	6.5	12.9	3.2						
22.5–45.0° (2)	9.7	16.1	12.9		3.2					
45.0–67.5° (3)	19.4	9.7	6.5							
67.5–90.0° (4)	16.1	29.0	25.8	16.1	3.2	3.2	6.5	3.2	3.2	3.2
90.0–112.5° (5)	19.4	12.9	19.4	19.4	25.8	22.6	19.4	16.1	6.5	6.5
112.5–135.0° (6)	12.9	6.5	3.2	19.4	22.6	19.4	25.8	32.3	41.9	45.2
135.0–157.5° (7)	6.5	6.5	9.7	22.6	22.6	35.5	22.6	22.6	22.6	19.4
157.5–180.0° (8)	3.2	3.2		12.9	12.9	6.5	12.9	16.1	12.9	12.9
180.0–202.5° (9)				3.2	9.7	6.5	6.5	3.2	6.5	6.5
202.5–225.0° (10)	3.2		3.2	3.2		6.5	3.2	3.2	3.2	3.2
225.0–247.5° (11)		3.2	3.2				3.2	3.2	3.2	3.2
247.5–270.0° (12)	3.2									
270.0–292.5° (13)										
292.5–315.0° (14)		3.2	3.2							
315.0–337.5° (15)		3.2								
337.5–360.0° (16)										
Hour 2										
0.0–22.5° (1)	3.2	9.7	3.2		3.2	3.2				
22.5–45.0° (2)	9.7	12.9	16.1				3.2			
45.0–67.5° (3)	16.1	6.5	9.7							
67.5–90.0° (4)	29.0	32.3	19.4	9.7	6.5	6.5	3.2	3.2	3.2	3.2
90.0–112.5° (5)	12.9	12.9	16.1	22.6	22.6	16.1	19.4	16.1	6.5	6.5
112.5–135.0° (6)	9.7	6.5	3.2	19.4	12.9	22.6	25.8	32.3	38.7	35.5
135.0–157.5° (7)	9.7	6.5	12.9	22.6	35.5	22.6	19.4	19.4	25.8	29.0
157.5–180.0° (8)		3.2		3.2	3.2	12.9	16.1	16.1	12.9	12.9
180.0–202.5° (9)				6.5	3.2	3.2				3.2
202.5–225.0° (10)	3.2		3.2	9.7	9.7	9.7	6.5	6.5	9.7	6.5
225.0–247.5° (11)		3.2	6.5	3.2	3.2	3.2	6.5	6.5	3.2	3.2
247.5–270.0° (12)			6.5							
270.0–292.5° (13)	3.2		3.2							
292.5–315.0° (14)		3.2								
315.0–337.5° (15)	3.2	3.2								
337.5–360.0° (16)										
Hour 3										
0.0–22.5° (1)	3.2	6.5	3.2							
22.5–45.0° (2)	16.1	12.9	3.2							
45.0–67.5° (3)	12.9	6.5	12.9							
67.5–90.0° (4)	29.0	25.8	16.1	6.5	6.5	6.5	9.7	3.2	3.2	3.2
90.0–112.5° (5)	9.7	16.1	22.6	25.8	16.1	16.1	16.1	12.9	6.5	6.5
112.5–135.0° (6)	9.7	6.5	12.9	12.9	19.4	25.8	29.0	35.5	41.9	38.7
135.0–157.5° (7)	9.7	12.9	12.9	29.0	32.3	22.6	19.4	22.6	22.6	19.4
157.5–180.0° (8)				6.5	6.5	9.7	9.7	12.9	12.9	22.6
180.0–202.5° (9)		3.2			3.2	3.2	3.2		3.2	
202.5–225.0° (10)	3.2		3.2	12.9	6.5	6.5	6.5	9.7	6.5	6.5
225.0–247.5° (11)		3.2	3.2	3.2	6.5	6.5	6.5	3.2	3.2	3.2
247.5–270.0° (12)			3.2							
270.0–292.5° (13)			3.2							
292.5–315.0° (14)	3.2	3.2	3.2							
315.0–337.5° (15)	3.2									
337.5–360.0° (16)		3.2			3.2	3.2				

Notes: Start height was 10 m; mean (±SD) downwind direction at hour 1 = 130° ± 55°. Trajectory positions are forward calculated with hour zero beginning at the control district center. EDAS data (National Weather Service's Eta Data Assimilation System) are generated every three hours; therefore the similarity between interpolated positions during hours 2 and 3 is not surprising. An approximation of the wind direction can be found by adding 180° to each area.

tail (Rogers 1993, Van de Water and Levetin 2001). Analysis of upwind trajectories from each location with sentinel and resident positive GM seedlings, showed specific days when air parcels passed over the control area prior to arrival at each location. Sentinel locations were deployed radiating away from the control area, therefore the number of trajectories crossing the control area prior to arrival at each positive location followed a decay curve, because pollen is subject to downwind deposition and impaction on vegetation (Jackson and Lyford 1999). The distance from the control area as well as a decline in the number of trajectories crossing each location showed that GM gene flow can be equated with declining atmospheric pollen concentrations for poten-

TABLE 2. Results of analysis of the position of the trajectory wind data and locations of the sentinel plants testing positive for GM seed progeny using top-down correlation (Iman and Conover 1987).

Time of day	Mean wind run (km/h)			Mean wind movement (km)			No. sites			r^2		
	Hour 1	Hour 2	Hour 3	Hour 1	Hour 2	Hour 3	Hour 1	Hour 2	Hour 3	Hour 1	Hour 2	Hour 3
06:00	4.9	5.1	5.3	4.9	10.0	15.3	9	47	69	**32.0**	5.5	5.9
07:00	5.0	5.2	5.5	5.0	10.2	15.7	10	49	71	**49.4**	2.1	0
08:00	5.2	5.6	6.1	5.2	10.7	16.8	11	50	72	19.0	**54.6**	1.8
09:00	6.0	6.0	6.5	6.0	12.1	18.6	16	60	72	2.1	**49.0**	**65.2**
10:00	7.5	7.5	8.1	7.5	15.0	23.1	32	69	73	3.1	**59.9**	**98.9****
11:00	9.1	9.2	9.8	9.1	18.4	28.2	45	72	74	**38.3**	0	7.6
12:00	11.1	11.1	11.5	11.1	22.1	33.7	51	74	75	0.1	24.9	**23.6**
13:00	13.0	12.8	13.1	13.0	25.8	38.9	65	75	75	5.9	4.9	4.9
14:00	14.7	14.3	14.5	14.7	29.0	43.5	69	75	75	0.1	2.2	0.1
15:00	16.1	15.7	15.7	16.1	31.8	47.5	71	75	75	0.3	0.1	0.1

Notes: The wind trajectory data were split into their hourly position (22.5° quadrants), and the wind run movement distance was calculated (km) for each hour over the 31-day pollination period. The positions of positive sites occurring within the distance of each hourly mean wind run were calculated. Top-down correlation (Iman and Conover 1987) used the four greatest occurrences of wind position vs. the number of positive sentinel seed progeny to generate correlation statistics. Low numbers of sites during the first hour results from short wind run distances. The center of the "control" area stretches ~8 km toward the south to southeast. Therefore, significant site numbers are not included until after 11:00 hours in hour 1 when the mean wind run movement distance exceeds 8 km/hr. High early morning correlations during hour 1 result from low site numbers and should be viewed as inconclusive. Correlation coefficients for the three highest values appear in bold type. Although only a single hourly comparison is statistically significant, winds crossing positive progeny sites during or just prior to the noon hour are indicated by the larger correlation factor values.

** $P < 0.01$.

tial fertilization (Fig. 3). Therefore, the most distant locations showing pollination by GM pollen were the most instructive with regard to the timing and the concentration of pollen arrival. In this case, the influence of the mean wind direction resulted in locations from the southeast quadrant having the greatest number of trajectories crossing the control area prior to arrival at the sentinel location positive for GM seeds, at any given distance. Locations deployed in the southwest and northeast quadrants had the second largest number of trajectories, respectively (Fig. 3). Locations in the northwest quadrant, showing GM pollination were limited to relatively few instances when winds from the control area moved toward them. Fertilization of plants in the northwest quadrant was ascribed to processes secondary to downwind dispersal (see *Discussion*).

Analysis of the most distant location shown to have progeny positive for the GM gene indicated winds blowing through the control area were limited to a three-day period. For four hours on 24 June and a single hour on 25 and 26 June, wind trajectories traveled south from the northwestern corner of the control area (Fig. 1).

TABLE 3. Percentage of sentinel plant positions occurring within designated bins (22.5°) surrounding the center of the Oregon Department of Agriculture's control district.

Area	*Agrostis stolonifera* non-detects†		Agrostis *stolonifera* positive detects‡		All sentinel plants (22.5°)	
	No. sentinel plants	Total (%)	No. sentinel plants	Total (%)	Total no. plants	By strata (%)
1) 0.0–22.5°	0	0.0	1	1.3	1	100
2) 22.5–45.0°	4	6.3	1	1.3	5	20
3) 45.0–67.5°	2	3.2	2	2.7	4	50
4) 67.5–90.0°	4	6.3	1	1.3	5	20
5) 90.0–112.5°	7	11.1	5	6.7	12	42
6) 112.5–135.0°	2	3.2	7	9.3	9	78
7) 135.0–157.5°	15	23.8	19	25.3	34	56
8) 157.5–180.0°	11	17.5	19	25.3	30	64
9) 180.0–202.5°	10	15.9	8	10.7	18	44
10) 202.5–225.0°	3	4.8	1	1.3	4	25
11) 225.0–247.5°	2	3.2	1	1.3	3	33
12) 247.5–270.0°	0	0.0	3	4.0	3	100
13) 270.0–292.5°	1	1.6	1	1.3	2	50
14) 292.5–315.0°	1	1.6	5	6.7	6	83
15) 315.0–337.5°	1	1.6	0	0.0	1	0
16) 337.5–360.0°	0	0.0	1	1.3	1	100

Notes: The last column shows the percentage of the total number of plants with GM-positive progeny in each area. Comparison with the non-detected *A. stolonifera* shows that areas 5–9 have the greatest number of plants.

† $N = 63$ plants.

‡ $N = 75$ plants.

TABLE 4. Percentage of resident *Agrostis stolonifera* and *A. gigantea* plant positions occurring within designated bins (22.5°) surrounding the center of the Oregon Department of Agriculture's control district.

	Agrostis stolonifera					*Agrostis gigantea*				
	Non-detects†		Positive detects‡			Non-detects§		Positive detects¶		
Area	No. plants	Total (%)	No. plants	Total (%)	All plants (%)	No. plants	Total (%)	No. plants	Total (%)	All plants (%)
1) 0.0–22.5°	0	0.0	0	0.0	0	0	0.0	1	7.7	100
2) 22.5–45.0°	0	0.0	0	0.0	0	1	3.8	0	0.0	0
3) 45.0–67.5°	3	21.4	3	18.8	50	0	0.0	0	0.0	0
4) 67.5–90.0°	0	0.0	0	0.0	0	0	0.0	0	0.0	0
5) 90.0–112.5°	1	7.1	0	0.0	0	1	3.8	0	0.0	0
6) 112.5–135.0°	1	7.1	1	6.3	50	1	3.8	3	23.1	75
7) 135.0–157.5°	3	21.4	0	0.0	0	5	19.2	3	23.1	38
8) 157.5–180.0°	2	14.3	6	37.5	75	3	11.5	2	15.4	40
9) 180.0–202.5°	3	21.4	1	6.3	25	8	30.8	2	15.4	20
10) 202.5–225.0°	1	7.1	0	0.0	0	2	7.7	1	7.7	33
11) 225.0–247.5°	0	0.0	1	6.3	100	1	3.8	1	7.7	50
12) 247.5–270.0°	0	0.0	0	0.0	0	0	0.0	0	0.0	0
13) 270.0–292.5°	0	0.0	0	0.0	0	0	0.0	0	0.0	0
14) 292.5–315.0°	0	0.0	2	12.5	100	1	3.8	0	0.0	0
15) 315.0–337.5°	0	0.0	1	6.3	100	2	7.7	0	0.0	0
16) 337.5–360.0°	0	0.0	1	6.3	100	1	3.8	0	0.0	0

Notes: Ten *Polypogon monspeliensis* plants collected throughout the area show no seeds testing positive for GM genes and are thus excluded. The last column for each species shows the percentage of the total number of plants with non-detect and GM-positive progeny in each area surrounding the control district.

† $N = 14$ plants.
‡ $N = 16$ plants.
§ $N = 26$ plants.
¶ $N = 13$ plants.

Analysis of measured wind direction at the Madras weather station showed strong correlations with measured winds after 10:00 hours on these three days. From 06:00 to 09:00 hours, measured winds shifted counter-clockwise and were consistently >90° off modeled winds. From 10:00 to 14:00 hours, winds were shifted clockwise and <30° off modeled wind directions (Fig. 2). In fact, winds on 24 June arrived at 89% of all

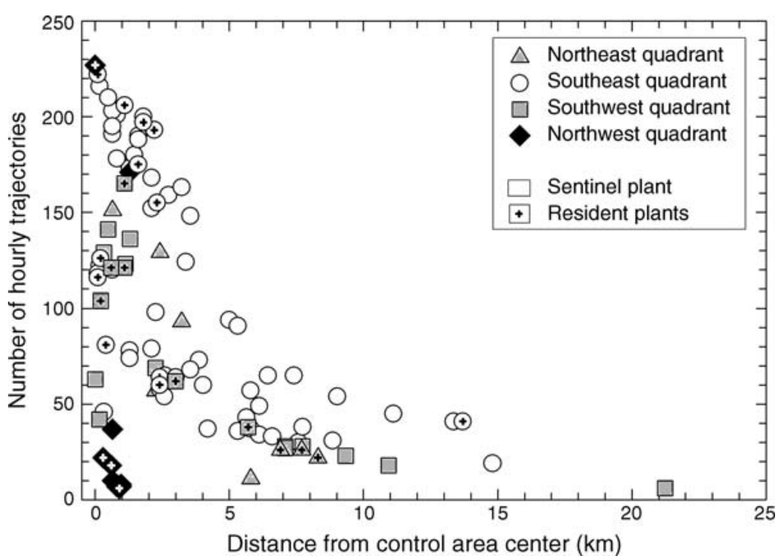

FIG. 3. The number of hourly trajectories between 16 June and 15 July 2003 that originated in the control area and arrived at each of 75 sentinel and 29 resident plant locations with GM-positive seedling progeny. Locations are grouped by their quadrant of occurrence and show an exponential decrease in the number of trajectories crossing each sentinel or resident position with respect to distance. Theory suggests trajectory probability at any downwind distance (r) should be proportional to $2\pi r$. The southeast quadrant and the mean wind direction show the greatest number of trajectories. Both the northeast and southwest quadrants have reduced trajectory crossing numbers. The northwest quadrant showed the fewest trajectory crossings. Locations in the northwest quadrant near the Deschutes River canyon occur upwind of the mean wind direction. Distances were measured from each location to the control area boundary for consistency with Watrud et al. (2004). Similar results were obtained when distances were calculated from the control area center.

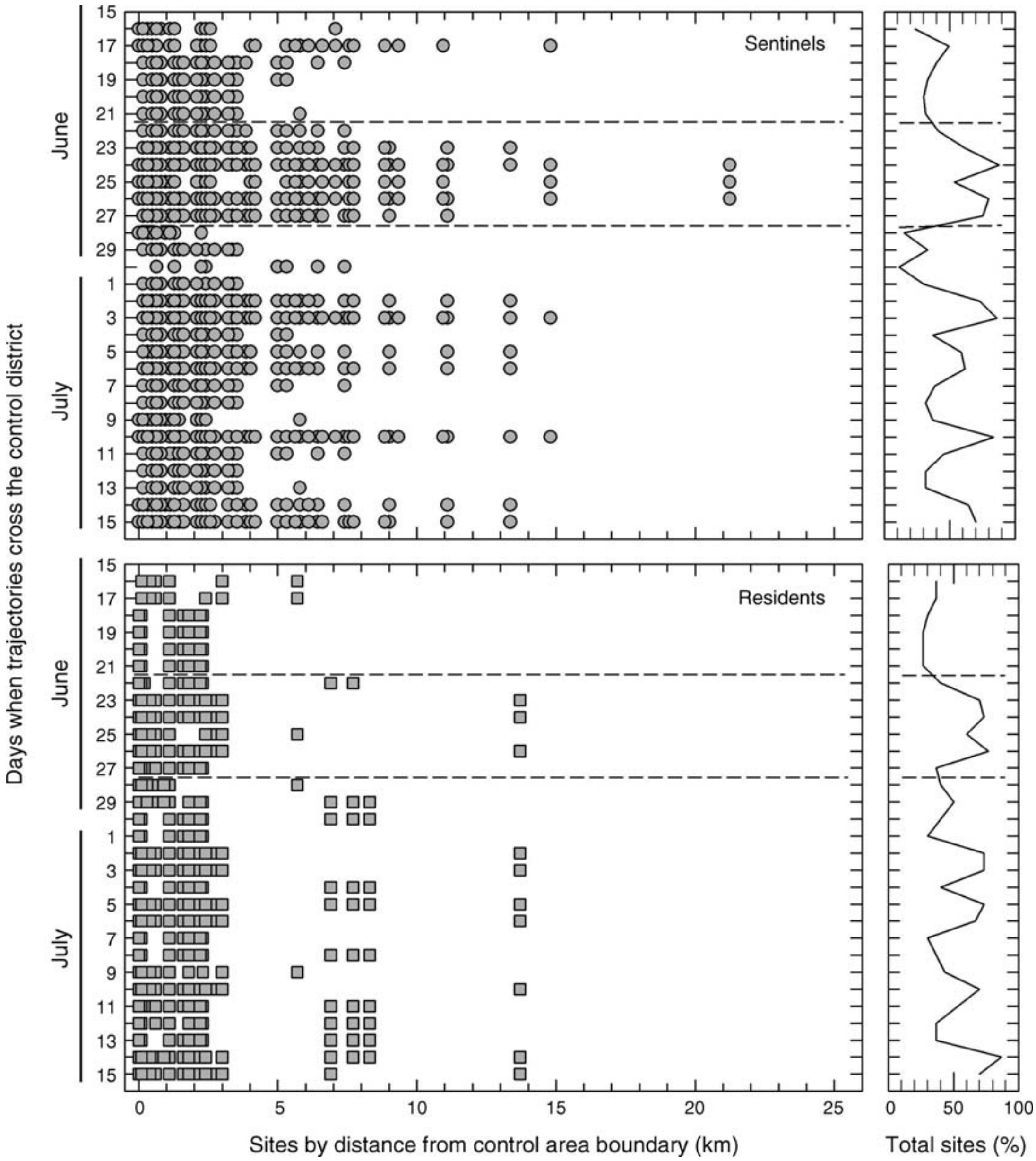

FIG. 4. Each point represents one of the 75 sentinel or 29 resident plant locations that produced GM-gene-positive seedling progeny that were crossed by at least one trajectory from the control area for that particular day in 2003. The plot clearly shows that locations closest to the control area had the greatest opportunity for downwind fertilization compared to those at greater distances.

downwind locations that resulted in positive progeny, after crossing the control area. If locations outside the control area but upwind of the mean wind direction were excluded, this rose to 97% of the locations (Fig. 4). A similar picture emerged with the resident plants. High percentages of trajectories crossed the control area prior to arriving at resident plant locations showing GM gene seedlings during this period. On 24 June, 75% of all resident locations were crossed by trajectories first traveling over the control area (Fig. 4). Finally, the timing of the greatest number of wind trajectories crossing the farthest sentinel location resulting in GM seedlings was consistent with active pollination on 24 June.

Pollen release does not occur on a single day, but over an extended period of time (days to weeks). The days before and after 24 June showed a significant number of locations positive for GM progeny being crossed by trajectory pathways traveling over the control area. Of all the sentinel and resident plant locations, only a single resident location positive for GM-gene seeds failed to be crossed by trajectories traveling over the control area prior to arrival. This location lays to the northeast of the control area and is one of three locations with GM gene seeds in a tributary canyon of the Deschutes River (Fig. 1). Analysis of the period from 22 to 27 June showed the percentage of sentinel locations positive for GM-gene progeny, where trajectories cross the control area prior to arrival, averaged 72%, ranging from 52% to 89% (Fig. 4). Similar high percentages, above 80%, were not reached again until 2 and 3 July, July 10, and again on 14 and 15 July. For the same period with respect to the resident plant locations whose seedlings tested positive for the GM gene, winds crossed the control area prior to arrival at 66% of the locations ranging from 38% to 79%. Percentages over 70% occurred again on 2 and 3 July, 5 July, and 10, 14, and 15 July. The period of 22 to 27 June had the most sustained period of trajectories crossing the control area prior to arrival at sentinel and resident plants showing GM progeny. This suggests the period from 22 to 27 June witnessed the greatest period of downwind (south-southeast) fertilization.

Additional information from field notes and environmental data showed moderate conditions during the hypothesized pollination period in late June. The sentinel plants were deployed on 17 and 18 June 2003, the first watering occurred during the first week of July. Daily high temperatures in the area between 22 and 27 June started around 16°C rising to around 32°C by 29 June 2003 (Fig. 5). Increased surface heating is consistent with increased variability between measured and modeled wind directions beginning in latest June. Precipitation was recorded as a trace on 18 June and 0.30 cm on 20 June. Cloudy conditions indicated increased diffuse solar radiation levels from 18 to 24 June changing to clearer skies and high solar radiation, later. Air trajectory and measured wind speeds showed diurnal as well as day-to-day fluctuations during this period. Average daily wind speeds were relatively low between ~2 and ~4 km/h on 24–26 June. Higher speeds, ~7 km/h occurred on 22, 23, and 27 June (Fig. 5, Table 2). During this period, winds increased from low levels in the morning to the highest speeds after noon. Throughout, hours with strong winds showed better correlations between modeled and measured wind directions, whereas calmer conditions showed greater differences.

Discussion

Field tests of GM-modified bentgrass (*A. stolonifera*) in the ODA control area during the 2003 growing season provided the means to test the significance of long-distance transport in this wind-pollinated crop plant. Modeled wind trajectories were generated as the mechanism of long-distance pollen flow to link specific atmospheric conditions to the occurrence of GM genes in plant progeny outside the ODA control area. Modeled winds showed a predominantly northwest to southeast wind direction during the summer of 2003. Sentinel and resident *A. stolonifera* and *A. gigantea* plant locations, whose progeny tested positive for the GM gene, are distributed around the control area. Yet, a significant percentage of the non-detect sentinel locations also lay along the mean wind direction. By back calculating trajectories from sentinel locations with GM-gene-positive seeds, active pollination most likely was occurring on 24 June 2003. This conclusion was based on the only occurrence of multiple wind trajectories in a single day crossing the control area prior to arrival at the farthest sentinel location. The high percentage of winds crossing the control area prior to arrival at the other locations with GM-gene-positive seeds suggested that the period 22–27 June was the most coherent and concentrated wind activities from 15 June to 15 July that could likely result in viable pollen transport and successful fertilization.

Long-distance nonviable pollen transport by wind is well known in non-GM species from the identification of unique pollen types outside their biogeographical range (Wynn-Williams 1991, Bourgeois 2000), or from the downwind arrival of a morphologically distinct pollen type during a known pollination period (Rogers and Levetin 1998, Van de Water and Levetin 2001, Van de Water et al. 2003). However, many crop plants, especially the grasses, have pollen that is essentially indistinguishable from related species contributing to the local and regional pollen rain, thus making the separation of causal agents for gene-flow at a landscape level difficult. Recent work on GM plants allows unique genetic signatures to provide unequivocal markers to detect gene flow to their progeny (Scheffler et al. 1995, Rieger et al. 2002, Wang et al. 2004*b*). In this study, the fertilization of sentinel and native species outside the ODA control area, during the initial year of testing GM bentgrass, showed significant long-distance pollen-mediated gene flow based on use of a selectable marker (Watrud et al. 2004). With a high potential for population establishment outside of the control area (Reichman et al. 2006), a better understanding of the processes involved in long-distance pollen-mediated gene-flow are needed to facilitate efforts to mitigate and model the potential spread of GM genes into wild plant communities.

For fertilization to occur, viable pollen must be delivered downwind to receptive plant stigmas. Overall, the longevity of grass pollen viability is considered to be low. For example, Fei and Nelson (2003) report *A. stolonifera* pollen viability had high fertility rates within an hour of shedding but within three hours it dropped to nearly zero. Our findings (Watrud et al. 2004 and the current study) are consistent with the distribution of positive GM-gene locations falling within the three-hour distance projected by modeled wind trajectories. The occurrence of more plants testing positive for GM genes in their seedling progeny closer to the control area boundary as compared to locations farther downwind is indicative of declining pollen concentration and viability and thus a reduced chance for fertilization with distance

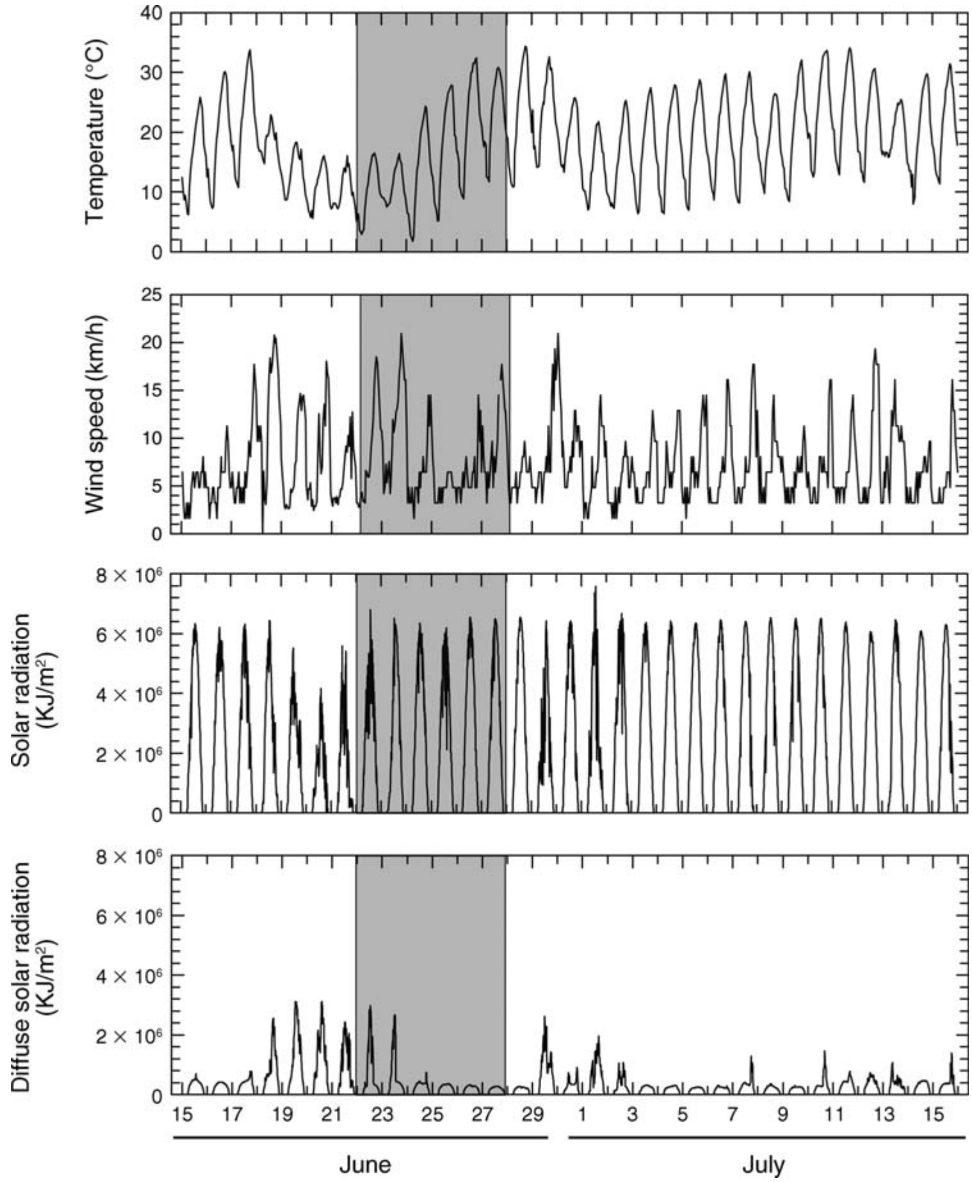

FIG. 5. Temperature (°C), wind speed (km/h), solar radiation (KJ/m^2), and diffuse solar radiation (KJ/m^2) levels for the 30-day period from 15 June to 15 July 2003 in Madras, Oregon, USA. An increase in diffuse solar radiation occurs during cloudy conditions at the expense of solar radiation. The period representing the maximum potential pollen release period, based on the highest percentage of total sentinel and resident sites crossed by trajectories (Fig. 4), is delineated by the gray box.

from the GM source fields. However, additional factors such as hourly changes in wind strength (Fig. 5), trajectory pathways (Fig. 3), and the timing of pollen shed also need to be considered relative to downwind movement. For example, Fei and Nelson (2003) report pollen viability peaks, regardless of when pollen shed begins, at 09:00 and 14:00 hours. This double peak occurs in the mid-morning and afternoon; periods characterized in this study by similar wind direction and increased wind strength. It is also a period of increased similarity between modeled and measured atmospheric wind direction. Our observation of higher correlations between wind and sentinel plant positions (Table 2) during the mid-morning period correspond with the early pollen viability peak, but not the afternoon peak.

Environmental conditions also affect pollen viability. For example, *Festuca arundinacea* pollen viability drops to less than 5% in 30 minutes and to zero after 90 minutes when sunny atmospheric conditions exist (Wang et al. 2004*a*). However, under cloudy conditions with cooler temperatures and reduced UV-B radiation, viability may extend up to 240 minutes with the 5% level reached after 150 minutes. It is reasonable to assume local weather and other environmental conditions in Madras affected the viability during long distance transport of GM pollen from the experimental fields to the downwind sentinel and resident locations. This is especially true during the afternoon when conditions were generally sunny and warm with higher wind speeds. The position of the farthest sample with GM-positive seedling progeny suggests pollen viability needed to be at least 2.5 h for successful entrainment and delivery (based on a distance of 21 km and average wind speeds of 9–11 km/h; Table 2). Field conditions just after sentinel plant emplacement were relatively cool and cloudy with precipitation recorded locally on 18 and 20 June. These weather conditions cleared over the following weeks as clear skies prevailed and high temperatures steadily climbed from ~15°C to over 30°C by the end of the month (Fig. 5). Sentinel plants were not watered until the week of 6 July, therefore the sentinel plants were under increasing water stress during this period.

GM gene transfer occurred more than 21 km away from the edge of the control area boundary (Watrud et al. 2004). Small scale studies routinely report crop to crop GM-gene flow distances in meters, not kilometers (Stringam and Downey 1978, Wang et al. 2004*b*). The single exception to date is the large field test of oilseed rape (*Brassica napus*) in Australia where gene flow of GM herbicide-resistant pollen was detected 3 km downwind (Rieger et al. 2002). Genetic out-crossing to native species is reported in a number of cases and at distances greater than the small scale crop-to-crop gene-flow studies but within 1–2 km of the pollen source (Kirkpatrick and Wilson 1988, Klinger et al. 1991, Arias and Rieseberg 1994). In this instance, out-crossing of the GM gene from *A. stolonifera* to *A. gigantea* occurred up to 14 km away from the control area boundary. Many small scale studies use limited source material, i.e., hundreds of plants and/or single small micro-plot source areas. In comparison, the acreage in this study consisted of 162 ha in the ODA control area composed of nine planted and eight flowering fields with about 2.8×10^6 seeds planted per hectare (estimated using a planting rate of 3 ounces per acre and 6×10^6 seeds per pound). Concentrated pollen source fields provide high pollen concentrations for downwind travel. In addition, fields are grown under homogeneous agricultural conditions that potentially reduce variability in the timing of pollen release during the period of anthesis. Even with constant loss along the way, a strong source supply delivers higher pollen concentrations at greater distances. In this case, if fertilization occurred on 24 June at 21 km, it followed a period of cool temperatures, lighter winds, and significant cloud cover over the area (Fig. 5). Whereas the environmental conditions on 24 June were not optimal for extended pollen viability, a large release caused by optimal release conditions existing for the first time during the pollination season could increase the potential for downwind fertilization at significant distances. Finally, local topography may also contribute as the most distant sentinel location is in a pass between higher elevations, where air masses moving south-southwest from the control area would compress as they funnel through.

Sentinel and resident plant locations resulting in GM-gene-positive progeny that occurred upwind of the mean wind direction suggested topographic influences on local wind conditions. The northwestern edge of the control district lies at the top of the Deschutes River Canyon. From the rim to the river, a lateral distance of <1.0 km, the canyon walls drops almost 300 m. Wind movements in this deep canyon are tied to regional processes of atmospheric exchange between eastern and western Oregon. These pressure differences are mediated through the Columbia River Gorge, with the Deschutes River Canyon acting as a feeder for winds moving either up or down canyon to equilibrate pressure differences east to west (G. Taylor, *personal communication*). In addition, feeder canyons of Willow, Sagebrush, and Mud Springs creeks occur to the southwest, north and northeast of the ODA control area. The measured morning (06:00–09:00 hours) ground-level wind direction at the Madras Airport showed values as great as 180° counterclockwise of the modeled wind trajectories from the northwest quadrant. Values are in agreement with winds moving out of and into the deeper canyons to the southwest, west, and northwest vs. the more stable and flat Agency Plains and rolling hills to the east and southeast (Figs. 1 and 2). The presence of these topographic features suggested ground-level wind direction and atmospheric characteristics potentially differed from regional trends and could draw potentially pollen laden air masses against the mean-wind direction, into and down-canyon where sentinel and resident plants with seeds testing positive for GM genes were found.

The spatial distribution of pollen-mediated GM gene flow can facilitate our understanding of long-distance pollen transport. The primary mechanism of pollen entrainment and subsequent travel results in fertilization and GM gene flow. However, winds associated with specific periods of viable pollen release should strongly influence the timing and direction this occurs. As this study demonstrated, local topographic features also played a role in determining GM gene flow. With wind dispersion, the distance from the source fields is critical for gene flow to occur; close proximity to pollen sources increased the number of trajectories that crossed the source field prior to arrival. GM gene flow observed at significant distances appears to be a cautionary tale about multiple factors, e.g., environmental conditions, pollen viability, and local topography, that individually or collectively impacted GM gene flow. It should also be noted that, while GM gene transfer was detected at 21 km, the experimental sampling design extended only to 24 km beyond the perimeter of the ODA control area. The HYSPLIT model predicted atmospheric transport of viable pollen, three hours downwind of the initial release point, as far as 75 km. The maximum potential spread of GM pollen observed may be an underestimate not only with regard to distance from the control district, but even more so from individual source fields of GM creeping bentgrass.

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