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RESPONSE OF AN ALLERGENIC SPECIES, *AMBROSIA PSILOSTACHYA* (ASTERACEAE), TO EXPERIMENTAL WARMING AND CLIPPING: IMPLICATIONS FOR PUBLIC HEALTH[1]

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We examined the responses of an allergenic species, western ragweed (*Ambrosia psilostachya* DC.), to experimental warming and clipping. The experiment was conducted in a tallgrass prairie in Oklahoma, USA, between 1999 and 2001. Warming increased ragweed stems by 88% when not clipped and 46% when clipped. Clipping increased ragweed stems by 75% and 36% in the control and warmed plots, respectively. In 2001, warming resulted in a 105% increase in ragweed aboveground biomass (AGB), and the ratio of ragweed AGB to total AGB increased by 79%. Dry mass per ragweed stem in the warmed plots was 37% and 38% greater than that in the control plots in 2000 and 2001, respectively. Although warming caused no difference in pollen production per stem, total pollen production increased by 84% ($P < 0.05$) because there were more ragweed stems. Experimental warming significantly increased pollen diameter from 21.2 μm in the control plots to 23.9 μm in the warmed plots (a 13% increase). The results from our experiment suggest that global warming could aggravate allergic hazards and thereby jeopardize public health.

Key words: aboveground biomass; allergy; *Ambrosia psilostachya* (Asteraceae); global warming; Oklahoma; pollen; ragweed; tallgrass prairie.

There are increasing risks to public health associated with global warming and changes in land use. Assessment of such risks poses a major challenge to scientists and policy makers. A main concern for public health highlighted in the *Third Assessment Report of the Intergovernmental Panel on Climate Change* (McCarthy et al., 2001) is allergic diseases, which each year affect more than 50×10^6 people and cost about 18×10^9 dollars for health care in the United States alone (AAAAI, 2000). Further, allergic diseases have increased among the general population over the last 50 yr (Platt-Mills and Carter, 1997; Woolcock and Peat, 1997; AAAAI, 2000), an increase that coincides with the unprecedented changes in the global environment since the 1950s. The prime cause of human allergies during the autumn is airborne ragweed (*Ambrosia*) pollen (Meggs et al., 1996), and the abundance of ragweed pollen is closely related to meteorological conditions (Buck and Levetin, 1982), suggesting that global warming may increase the amount of ragweed pollen and therefore human allergies. The responses of allergenic species, in particular their pollen production, to global warming, however, have not been carefully studied.

This study was designed to examine the effects of experimental warming and clipping on western ragweed (*Ambrosia psilostachya* DC.), an allergenic species. It is a part of a comprehensive warming experiment in a tallgrass prairie in the U.S. Great Plains (Luo et al., 2001; Wan, Luo, and Wallace, 2002). We used infrared heaters to mimic climate warming, and we clipped the plot to simulate mowing for hay, a widespread land-use practice in the tallgrass prairie of the Great Plains.

MATERIALS AND METHODS

Site description—The experimental site is located at the Great Plain Apiaries (34°58′54″ N, 97°31′14″ W), 40 km from the Norman campus of the University of Oklahoma. The site has not been grazed for the past 20 yr. The grassland is dominated by C_4 grasses (*Schizachyrium scoparium* (Michx.) Nash-Gould, *Sorghastrum nutans* (L.) Nash, and *Eragrostis curvula* (Schrad.) Nees) and C_3 forbs (*Ambrosia psilostachya* and *Aster ontarionis* Wieg.). Mean annual temperature is 16.3°C with a monthly mean temperature of 3.3°C in January and 28.2°C in July. The annual precipitation is 914 mm. The soil is part of the Nash-Lucien complex, which is characterized by having a low permeability, high available water capacity, and deep, moderately penetrable root zone (USDA Soil Conservation Service and Oklahoma Agricultural Experiment Station, 1963).

Experimental design—The experiment used a paired factorial design, with warming as the main factor nested with clipping. There were five pairs of 2×2 m plots. One plot in each pair had been warmed continuously with infrared heaters since 21 November 1999, and the other plot was the control. In each warmed plot, a single 165×15 cm infrared heater (Kalglo Electronics, Bethlehem, Pennsylvania, USA) was suspended 1.5 m above the ground. The heater had a radiation output of approximately 100 W/m^2. In the control plot, a ‘‘dummy’’ heater of the same shape and size as the infrared heater was suspended 1.5 m above the ground to simulate the shading effects of the heater. The distance between the control and warmed plots in each pair was approximately 5 m to prevent heating of the control plot by the infrared heater. The distances between the individual sets of paired plots varied from 20 to 60 m.

Each 2×2 m plot was divided into four 1×1 m subplots. Two diagonal subplots in each plot were clipped to 10 cm above the ground on 15 November 1999, 28 July 2000, and 24 July 2001; the other two were the unclipped control subplots. Clipping removed approximately 85% of the aboveground biomass. After clipping, plants were allowed to grow until the next clipping. The four treatments in the experiment were (1) unclipped unwarmed, (2) unclipped warmed, (3) clipped unwarmed, and (4) clipped warmed.

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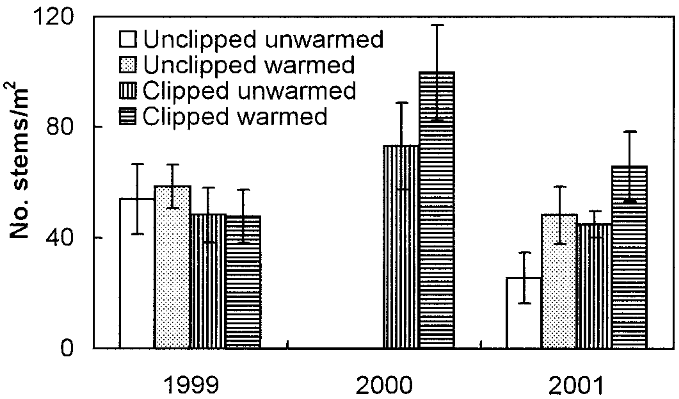

Fig. 1. Warming and clipping effects on western ragweed (*Ambrosia psilostachya*) stem numbers (means ± 1 SE).

Stem number, aboveground biomass, percent coverage—Ragweed stems were counted in the field on 25 August 1999 and 19 July 2001 and in the plant material clipped in 2000 and 2001. On 20 November 2001, living stems were counted in the unclipped subplots.

After clipping, plants were separated into ragweed, other forbs, and grasses, and each group was oven dried at 60°C for 48 h and then weighed. Ragweed aboveground biomass (AGB), the ratio of ragweed AGB to total AGB, and the dry mass per ragweed stem were measured and calculated.

In November 2000 and in April and June 2001, the percent coverage of ragweed was estimated using the point count method (Floyd and Anderson, 1987). A one-tailed paired *t* test was used for statistical analysis.

Pollen analysis—In September and October 2001, six ragweed stems in the unclipped, unwarmed subplots and the unclipped, warmed subplots in each pair were randomly selected for pollen sampling. Ragweed pollen was sampled once every 3 d from 21 September to 20 October 2001. Ragweed flowers were put into 17 × 20 cm zip-lock plastic bags (S. C. Johnson and Son, Racine, Wisconsin, USA) and shaken for 20 s. Pollen was washed from each bag with 20 mL of water. The pollen solution was put into culture plates with a diameter of 8.65 cm. The pollen in each sample was counted 15 times using an ocular micrometer under a light microscope (200×). The diameters of 42 pollen grains sampled on 6 October 2001 from both unwarmed and warmed plots were measured with an objective micrometer (320×). Pollen production for each date was calculated by multiplying the mean value of pollen counted within the range of the ocular micrometer by the quotient of the area of culture plate and the area of the ocular micrometer. Pollen production per stem was the quotient of the sum of pollen production for all sampling dates and the number of stems sampled. Total pollen production per subplot was calculated by multiplying the average pollen production per stem in the unwarmed and warmed plots by stem numbers in each subplot.

RESULTS AND DISCUSSION

Experimental warming significantly increased daily mean air temperature (up 1.2°C) and daily mean soil temperatures (up 1.8° and 2.7°C without and with clipping, respectively). Clipping also increased daily mean soil temperatures by 0.4° and 1.2°C in the unwarmed and warmed plots, respectively.

Experimental warming progressively increased the number of ragweed stems. Before the treatments in 1999, stem numbers were similar in the four subplots. Warming increased the number of stems slightly, but not significantly, in the clipped subplots (37%, $P > 0.10$) in 2000. By 2001 the number of stems in the warmed plots was significantly higher both without clipping (88%, $P < 0.05$) and with clipping (46%, $P < 0.05$) than in the unwarmed plots. Clipping also increased stem numbers by 75% ($P < 0.05$) in the unwarmed plots and by 36% ($P < 0.10$) in the warmed plots in 2001 (Fig. 1).

TABLE 1. Effects of warming on western ragweed (*Ambrosia psilostachya*) characteristics (means ± 1 SE) in clipped subplots of tallgrass prairie.

Characteristic	1999	2000	2001
Dry mass per stem (g)			
Unwarmed	0.57 ± 0.179	0.54 ± 0.054	0.37 ± 0.052
Warmed	0.41 ± 0.121	0.75 ± 0.078	0.52 ± 0.066
t test	0.2455	0.0343*	0.0288*
Aboveground biomass (AGB, g/m^2)			
Unwarmed	22.0 ± 5.89	43.3 ± 13.4	17.2 ± 2.77
Warmed	21.6 ± 8.70	69.0 ± 7.45	35.2 ± 8.29
t test	0.4887	0.1082	0.0150*
Ragweed AGB/total AGB (%)			
Unwarmed	4.31 ± 0.66	17.3 ± 3.39	12.2 ± 2.72
Warmed	4.99 ± 2.36	27.2 ± 4.34	21.9 ± 5.41
t test	0.3927	0.1131	0.0096**

* Statistically significant at confidence level of 95%.
** Statistically significant at confidence level of 99%.

Biomass production of ragweed was also stimulated by experimental warming. Dry mass per ragweed stem in the warmed plots increased significantly (by 37% and 38% in 2000 and 2001, respectively) compared with that in the unwarmed plots. Increased stem numbers and dry mass per stem in 2001 led to substantial increases in ragweed aboveground biomass per unit ground area (105%) and in the ratio of ragweed AGB to total AGB (79%) in the warmed plots compared with those in the unwarmed plots (Table 1).

Percent coverage of ragweed was 195% and 136% higher in the warmed plots than in the unwarmed plots in April and June 2001, respectively. A severe frost in November 2000 killed all ragweed stems in the unwarmed plots, whereas most stems survived in the warmed plots (Fig. 2a). After the first frost in November 2001, the number of living stems was significantly greater (239%) in the warmed plots (18.7 stems/m^2) than in the unwarmed plots (5.5 stems/m^2, Fig. 2b). The increase in percent coverage likely resulted from stimulated emergence and growth in spring and enhanced survivorship in fall under warming, suggesting an increase in the length of the growing season.

Increased stem numbers, biomass production, and percent coverage of ragweed in tallgrass prairie under warming may be attributable to several factors. First, well adapted to disturbed, dry, warm areas, ragweed has a higher photosynthetic rate than other forbs, partly because its taller stature gives it an advantage in light competition (Abul-Fatih and Bazzaz, 1979a, b; Bazzaz and Carlson, 1979). Second, experimental warming and increased light level at the soil surface due to clipping may stimulate germination of ragweed (Pickett and Baskin, 1973), resulting in increased stem numbers. Third, warming extends growing-season length, enhances survivorship, and promotes growth of ragweed. Fourth, ragweed has a high nitrogen requirement and shows a positive response to nitrogen fertilization (Hunt and Bazzaz, 1980; Vitousek, 1983). Thus, increased nitrogen availability under experimental warming (Rustad et al., 2001) may also indirectly enhance growth of ragweed. Moreover, the root exudates, leaf leachate, and decomposed litter of western ragweed release secondary metabolites that inhibit other species, such as *Andropogon ternaries* (Neill and Rice, 1971). Experimental warming may

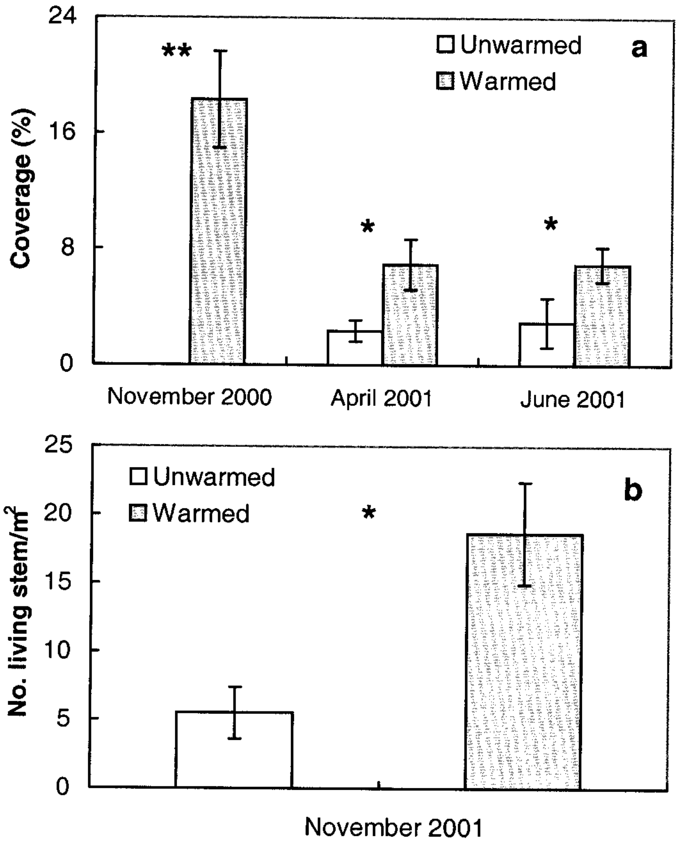

Fig. 2. Warming effect on (a) the percent coverage (means ± 1 SE) and (b) the number (means ± 1 SE) of living western ragweed stems in the unclipped subplots. *Statistically significant at confidence level of 95%, **statistically significant at confidence level of 99%.

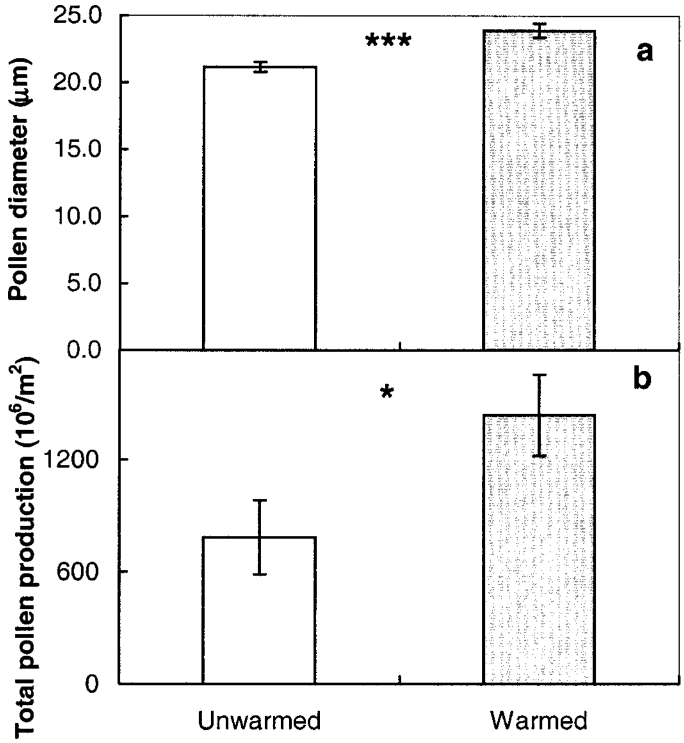

Fig. 3. Warming effects on (a) the diameter (means ± 1 SE) of pollen grains and (b) the total pollen production (means ± 1 SE) of western ragweed in the unclipped subplots. ***Statistically significant at confidence level of 99.9%, *statistically significant at confidence level of 95%.

augment such an allelopathic effect. Interactions of the above physiological and ecological processes may provide ragweed with a competitive advantage over other species in the tallgrass prairie community.

The diameter of the pollen grains was significantly greater (13%) in the warmed plots (23.9 μm) than in the unwarmed plots (21.2 μm, Fig. 3a). Allergenic proteins are located in both pollen walls (Howlett, Knox, and Heslop-Harrison, 1979) and cytoplasm (Grote, 1991, 1999; Rodríguez-Garcia, Fernández, and Alché, 1995; Staff, Schäppi, and Taylor, 1999). As a result, larger pollen grains could presumably carry more allergenic proteins than smaller ones. In addition, previous studies indicated that higher temperature increases protein and allergen content of pollen grains (Van Herpen, 1981; Hjelmroos, Schumacher, and Van Hage-Hamsten, 1995). Thus, enhanced pollen size and allergen content could lead to an increased allergic effect in humans. However, larger pollen grains may sink faster and travel shorter distances than smaller ones.

Total pollen production per unit of ground area increased significantly (84%, Fig. 3b) with warming, even though there was no difference in pollen production per stem between the warmed and unwarmed plots (data not shown). The increase in pollen production in the warmed plots resulted primarily from the increase in stem numbers. Our results are corroborated by palynological studies in which ragweed pollen abundance from lake sediment cores increased in dry, warm intervals during the postglacial period (Davis, 1969) and over the past 50 000 yr (Grimm et al., 1993).

Unprecedented changes in the global environment since the 1950s have coincided with increases in allergies among the general population (Platt-Mills and Carter, 1997; Woolcock and Peat, 1997; AAAAI, 2000), suggesting that global change might already be increasing the production of ragweed pollen. During the past 50 yr, atmospheric CO_2 concentration has increased by more than 20% (McCarthy et al., 2001). When the concentration of CO_2 was elevated experimentally from 280 to 370 ppm, ragweed pollen production increased by 131%; elevation of CO_2 from 370 to 600 ppm yields 90% more pollen (Ziska and Caulfield, 2000a, b). A recent study also showed that a doubling of the atmospheric CO_2 concentration from 350 to 700 ppm stimulated ragweed pollen production by 61% (Wayne et al., 2002). In addition to an increase in atmospheric CO_2 since the 1950s, global mean temperature has risen by approximately 0.5°C and extended the growing season by about 3 wk (Peñuelas and Filella, 2001). We found that an elevation in air temperature by 1.2°C and its associated change in the length of the growing season increased ragweed pollen production by 84%, suggesting that an intensified and lengthened allergic season might result. Elevated temperature increases not only allergenic pollen production but also protein and allergen content of pollen grains (Van Herpen, 1981; Hjelmroos, Schumacher, and Van Hage-Hamsten, 1995). Land-use has also changed greatly in the past 50 yr (McCarthy et al., 2001). Data from a palynological study (Russell, 1980) and a model simulation (Emberlin, 1994) indicate that land-use change affects ragweed pollen abundance. Our results also show that clipping, which mimics harvesting hay, stimulates growth, stem numbers, and total pollen production of ragweed. In short, the three concurrent driving forces of global change—rising atmospheric CO_2 concentration, global warming, and land-use change—may augment pollen production of ragweed and intensify cross-reactive allergies with other pollens and air pollutants (Staff, Schäppi, and Taylor, 1999; AAAAI, 2000; Epstein, 2000; Cifuentes et al., 2001; Fernandez et al., 2001).

Global change has the potential not only to exacerbate human allergies but also to increase the incidence of other diseases related to heat waves, flooding, drought, air pollution, and reduced food supply, as well as vector-, food-, and waterborne infections (Cohen, 2000; Epstein, 2000; Cifuentes et al., 2001; Fernandez et al., 2001; McCarthy et al., 2001). With a continuing increase in global mean temperature, airborne pollen abundance and other health risk factors will likely increase further. Our results and those from previous studies (Van Herpen, 1981; Hjelmroos, Schumacher, and Van Hage-Hamsten, 1995; Ziska and Caulfield, 2000a, b; Wayne et al., 2002) point to the urgency of reducing the emission of greenhouse gases. Mitigation of greenhouse gases will not only slow global warming but also substantially benefit public health (Cifuentes et al., 2001).

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