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Hygroscopic weight gain of pollen grains from *Juniperus* species

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Abstract *Juniperus* pollen is highly allergenic and is produced in large quantities across Texas, Oklahoma, and New Mexico. The pollen negatively affects human populations adjacent to the trees, and since it can be transported hundreds of kilometers by the wind, it also affects people who are far from the source. Predicting and tracking long-distance transport of pollen is difficult and complex. One parameter that has been understudied is the hygroscopic weight gain of pollen. It is believed that juniper pollen gains weight as humidity increases which could affect settling rate of pollen and thus affect pollen transport. This study was undertaken to examine how changes in relative humidity affect pollen weight, diameter, and settling rate. *Juniperus ashei*, *Juniperus monosperma*, and *Juniperus pinchotii* pollen were applied to greased microscope slides and placed in incubation chambers under a range of temperature and humidity levels. Pollen on slides were weighed using an analytical balance at 2- and 6-h intervals. The size of the pollen was also measured in order to calculate settling rate using Stokes' Law. All pollen types gained weight as humidity increased. The greatest settling rate increase was exhibited by *J. pinchotii* which increased by 24 %.

Keywords Hygroscopic · Pollen · Juniper · Settling rate · Terminal velocity · Mountain cedar

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Introduction

The Cupressaceae is a significant source of airborne allergens, and the genus *Juniperus* is a major component of many ecosystems across the northern hemisphere (Mao et al. 2010; Pettyjohn and Levetin 1997). New Mexico, Texas, and Oklahoma are home to many species of juniper. Three species that represent a significant allergy contribution are *Juniperus ashei*, *Juniperus monosperma*, and *Juniperus pinchotii*. *J. ashei* pollen is considered the most allergenic species of Cupressaceae in North America (Rogers and Levetin 1998). This species is distributed throughout central Texas, Northern Mexico, the Arbuckle Mountains of south central Oklahoma, and the Ozark Mountains of northern Arkansas and southwestern Missouri and pollinates from December to February (Pettyjohn and Levetin 1997; Adams 2008). *J. monosperma* is distributed throughout south central Colorado, much of New Mexico, Arizona, the pan handle of Oklahoma, and the pan handle of Texas as well as south western Texas (Adams 2008) *J. monosperma* is known to show cross-reactivity with *J. ashei* (Schweitz et al. 2000); this species pollinates from late winter to early spring (February–April) (Adams 2008). *J. pinchotii* is also cross-reactive with *J. ashei* and endemic to the south central United States, in central and western Texas, and adjacent Southwest Oklahoma, New Mexico, and Coahulia Mexico (Schweitz et al. 2000; Eckenwelder 2009). It is an important aeroallergen which pollinates from late September to late November (Pettyjohn and Levetin 1997; Weber and Nelson 1985; Wodehouse 1971).

The pollen from these species is produced in quantities of up to 523 billion pollen grains per tree (Bunderson et al. 2012). In addition, the pollen grains are small and lightweight and can be transported over great distances (Levetin and Buck 1986; Rogers and Levetin 1998; Levetin 1998; Levetin and de Water 2003). The distance that pollen travels after release depends on the pollen type and meteorological conditions. Although the majority of pollen released is deposited near

the source, a small percentage of pollen "escapes." The percentage of pollen or spores that is transported high enough in the atmosphere such that it is beyond the risk of immediate dry deposition on the ground is known as the "escape fraction" (Gregory 1978). Particles remain suspended as long as the upward movement of air is faster than the terminal velocity of the particle (Gregory 1973). The terminal velocity of suspended particles is affected by the environmental conditions of the air masses they encounter. For example, it is well known that air temperature, humidity, and pressure can affect the density and viscosity of air which can, in turn, affect settling rate (Tsilingiris 2008; Gregory 1978).

Relative humidity fluctuations can also affect pollen terminal velocity due to hygroscopic weight gain. Change in weight of pollen across a range of relative humidity levels has been estimated for various species. For example, four *Poa* species gained weight when exposed to a range of humidity levels (Diehl et al. 2001). Often, in response to fluid loss, pollen grains will accommodate change in cellular volume by folding inward on apertures during desiccation and unfolding during hydration (Wodehouse 1935). This folding and unfolding mechanism is referred to as harmomegathy (Wodehouse 1935). Although juniper pollen grains do not have a furrow and only have a very small pore, samples of the juniper pollen grains collected in this study were observed at collection and were in a non-spherical, desiccated state.

Pollen size, density, and settling rate have been estimated and calculated using a variety of methods. For example, Gilissen (1977) used a controlled humidity environment in an enclosed chamber to expose pollen to a desired temperature and humidity level and after which, the pollen was measured to determine the effects of the exposure. Durham (1946) estimated the settling rate of pollen by timing its travel through a 1-m tube. Settling rate is also commonly estimated using Stokes Law (Gregory 1973). Corn pollen density was calculated by measuring settling rate in uniform fluids with known specific gravity and viscosity (van Hout and Katz 2004). Once settling rate is established, pollen density can be estimated using Stokes' flow equation and this method requires no prior knowledge of particle size (van Hout and Katz 2004). Other methods require the measurement of pollen size in order to estimate pollen density. One method employs a gas pycnometer to determine particle density. A gas pycnometer can be used to measure the volume of a sample of many particles (e.g., pollen grains) by compressing a gas into the container holding the particles. The volume occupied by the particles is calculated based on the known properties of the gas and the expected amount of gas in the chamber minus the actual amount of gas in the chamber. This method has been used for corn pollen and ragweed pollen but the method is limited because it only measures the solid portion of the pollen grain (Harrington and Metzger 1963; Aylor 2002). Air that may have seeped into the pollen grain, altering the size and shape of the pollen, is not properly accounted for (van Hout and Katz 2004). Another method for estimating particle density requires an electrodynamic balance apparatus (EDB) which uses a net charge and a synthetic air environment to suspend particles. An EDB was used to measure the changing mass of *Sailx*, *Betula*, and *Narcissus* pollen (Pope 2010). The average increase in mass due to water uptake at 75 % humidity was 16 % (Pope 2010).

There is no information on how changing relative humidity levels affect the weight of Cupressaceae pollen. However, it is known that the thick cellulose-rich intine of Cupressaceae pollen is highly absorbent and will, in solution, absorb moisture and swell until the thin inflexible exine is shed (Takaso and Owens 2008). This swelling and shedding action is an important step in the pollination process when it occurs while suspended in the pollination drop. The pollination drop is a solution secreted by the ovule that acts as a kind of "liquid stigma," collecting pollen from the air (Dörken and Jagel 2014). Once the pollen has landed on a pollination drop, fluid is thought to flow through a very small pore in the exine which is plugged with a temporary structure, often called an operculum (Duhoux 1982). As the intine swells, it sheds the exine in a matter of minutes and depending on the solution, the intine itself breaks hours or days later releasing the protoplast (Chichiriccò and Pacini 2008). On a compatible ovule when the non-elastic exine is shed, the remaining portion of the pollen is flexible and travels more easily through the micropyle and micropylar canal (Takaso and Owens 2008). The absorption of water by the intine could also affect the flight of airborne pollen. If water vapor were absorbed through the exine it would affect the airborne transport of the pollen by changing the weight, size, and shape of the pollen grains. Though the amount of weight gain is unknown, *Cupressus arizonica* pollen has been shown to shed its exine at 100 % relative humidity between 6 and 24 h (Chichiricco et al. 2009).

Most pollen is released from anthers or microsporangiate cones during the daytime, and pollen concentrations are often negatively correlated with relative humidity (Weber 2003). Glassheim et al. (1995) suggest that the reason for this is that moist air inhibits pollen dispersal due to hygroscopic weight gain and interference with floret drying and anther separation. *J. ashei* pollen requires relative humidity below 50 % and temperature above 5 °C with dry conditions persisting for 24 h (Levetin and de Water 2003). Despite the required conditions for release, juniper pollen can come into contact with a wide variety of temperatures and humidity levels because it can be suspended in the air for long periods (Van de Water and Levetin 2001). Knowing how much hygroscopic weight gain juniper pollen experiences across the range of possible humidity levels could elucidate the degree of the humidity effect on pollen settling rates and inform researchers interested in conducting dispersion modeling studies. This experiment was designed to test the magnitude of the effect of changing relative humidity on pollen weight, size, and settling rate. It is expected that as relative humidity increases, weight and size will also increase.

Materials and methods

Pollen

J. ashei, *J. monosperma*, and *J. pinchotii* pollen were collected from native populations and refrigerated at 4 °C until used. The number of pollen grains per milligram was determined using the hemocytometer method. Two milligrams of *J. ashei* pollen was suspended in 1.5 ml of FAA, and 2.5 mg *J. monsperma* and *J. pinchotii* pollen were suspended in 1 ml of FAA. Approximately 10 μl of the suspension was placed on each side of the hemocytometer. The number of pollen grains was estimated using standard hemocytometer dilution conversions, and the counts were repeated six times (Pettyjohn and Levetin 1997).

Humidity experiments

Pollen were exposed to different levels of relative humidity inside desiccation chambers and evaluated after exposure similar to that of Gilissen (1977) and Chichiricco et al. (2009). Ten microscope slides (pollen slides) were coated with Lubriseal stopcock grease (Thomas Scientific) and dusted with pollen using a 1-ml syringe. Ten grease-coated but non-pollen-dusted slides (greased) and ten non-greased, non-dusted slides (ungreased) were used as controls. The ungreased slides were used to visually track condensation on the slides in order to eliminate condensation as a factor. Treatments with apparent condensation were eliminated from analysis and rerun.

Two 30×30×30 cm polyurethane desiccation boxes were placed inside a climate-controlled growth chamber (Percival Industries, Atlanta, GA) or a walk-in cold room (4 °C treatment) and used to expose the pollen-coated slides to various temperatures and humidity levels. In order to achieve a wide range of humidity levels, two humidity control methods were employed: dry silica gel and saturated salt solutions (Connor and Towill 1993). For 20 and 40 % humidity levels (Art Preservation Services, Long Island City, NY) and 50 and 60 % humidity levels (Heartfelt Industries), dry silica gel was used. In order to achieve 76 % humidity, 300 ml of a saturated NaCl solution (>0.37 g/ml) was used. For 85 % humidity, 300 ml of saturated KCl (>0.40 g/ml) was used. Ninety-seven percent humidity was achieved with 300 ml of a saturated K_2SO_4 (>0.12 g/ml) solution (Rockland 1960). Two hygrometers were placed at different elevations inside both desiccation boxes in order to monitor humidity.

Pollen-dusted slides were standardized by incubation at 20 °C and 60 % humidity for at least 12 h and then placed in the second desiccation box for the desired experimental humidity and temperature and measured at 2- and 6-h intervals. The 2-h time interval was chosen because it was the minimum time needed for the chambers to equilibrate for the low humidity levels and 6 h was chosen because it was the minimum time required for *C. arizonica* exine shed at 100 % relative humidity (Chichiricco et al. 2009). New pollen slides were created for each temperature and humidity level. All slides were weighed using an analytical balance. Humidity of the saturated salt solutions varied slightly with temperature. Humidity treatments will be referred to in terms of the relative humidity (RH) expected at 20 °C to eliminate confusion. A detailed breakdown of actual humidity at a given temperature and salt solution combination is provided (Table 1). *J. ashei* and *J. monosperma* pollen were exposed to 20, 40, 50, 76, 85, and 97 % humidity at 20 °C. *J. ashei* was also exposed to 20, 40, 76, and 97 % at 15 °C. *J. pinchotii* was exposed to 20, 40, 76, and 97 % at 20 °C. For *J. ashei*, an additional temperature of 4 °C was achieved in a walk-in cold room using humidity levels of 20, 40, 50, 76, 85, and 97 % (Table 1). Limited pollen inventory prevented all juniper species from being exposed to all levels of humidity and temperature and from further replication. A pollen slide was analyzed after each 6-h humidity exposure treatment in order to determine which, if any, humidity levels caused the exine to be shed. This was achieved by adding a drop of immersion oil and a cover slip to the pollen slide (Pacini et al. 1999). At least 100 pollen grains were counted in a traverse of the microscope slide.

Settling rate

Stokes Law was used to estimate the effect of the change in humidity on settling rate (terminal velocity) of *J. ashei* pollen. Stokes Law estimates the velocity of sedimentation (v_s) for a spherical particle: $v_s = \frac{2}{9}\frac{(P_p - P_f)}{\mu} g r^2$ (Gregory 1973). The density and viscosity of air varies with elevation, water content, and temperature. In order to complete the calculations, the following variables were used: g (981 cm/s), P_p (pollen density (g/cm^3)), P_f (density of air (g/cm^3)), and μ (viscosity of air (g/cm/s)) as calculated using the equations from Tsilingiris (2008) and for simplicity, barometric pressure was assumed to be 1 atm.

Table 1 Temperature and humidity levels for *J. ashei*, *J. monosperma*, and *J. pinchotii*

Treatment	*J. ashei*			*J.monosperma*	*J.pinchotii*
Rel. humidity	20 °C	15 °C	4 °C	20 °C	20 °C
20 %	20 %	20 %	20 %	20 %	20 %
40 %	40 %	40 %	40 %	40 %	40 %
50 %	50 %	ND	50 %	50 %	ND
76 %	76 %	76 %	75 %	76 %	76 %
85 %	85 %	ND	88 %	85 %	ND
97 %	97 %	99 %	98.5 %	97 %	97 %

ND no data available

Pollen diameter was determined using the following procedure: Three pollen-dusted slides were placed in 20 °C and 20 % RH conditions for 6 h, and 3 dusted pollen slides were placed in 20 °C and 97 % RH conditions for 6 h. Both humidity levels were repeated at 4 °C for *J. ashei* only. After the allotted time, a drop of immersion oil was added to the pollen on each slide (Pacini et al. 1999). A cover slip was added and the slide was immediately photographed at ×400. At least 100 pollen grains were measured for each *J. ashei* treatment and 30 pollen grains for each *J. monosperma* and *J. pinchotii* treatment. Images of pollen were analyzed to determine pollen cross-sectional area using ImageJ, and diameter was calculated using $d = \sqrt{4A_p/\pi}$ where A_p is the projected area of the pollen grain (van Hout et al. 2008). Pollen volume was also calculated and combined with pollen weight values to determine pollen density.

Statistical analysis

A two-way repeated measures ANOVA analysis was performed for *J. ashei*, *J. pinchotii*, and *J. monosperma* using time as the repeated factor and slide treatment (pollen slide/greased control/ungreased control) and humidity as additional factors. In the case of *J. ashei*, temperature was an additional factor. Since not all levels of humidity were used for the three levels of temperature, temperature was analyzed separately in a two-way ANOVA using the levels of temperature and slide treatment. Humidity and pollen weight gain were also tested independently using simple regression. In addition, a *t* test was used to compare pollen diameter at 97 % RH and 20 % RH for *J. monosperma* and *J. pinchotii* and a two-way ANOVA was used to compare the *J. ashei* treatments (SAS JMP 2010).

Results

The average weight per pollen grain as measured using a hemocytometer after incubation at 60 % RH was 2.5×10^{-6} mg, 4.0×10^{-6} mg, and 4.8×10^{-6} mg for *J. ashei*, *J pinchotii*, and *J. monosperma*, respectively. Pollen weight was positively correlated with humidity for all species and at all temperatures (Figs. 1, 2, and 3). In addition, weight change was usually negligible between 2- and 6-h intervals. Exine shed was also negligible. No less than 98 % of pollen grains had their exines intact before humidity treatment for each juniper species at any given humidity level, and the same percentage existed after the 6-h treatment. If the high humidity treatments caused exine shed at the 6-h juncture, our sample size was not large enough to detect this.

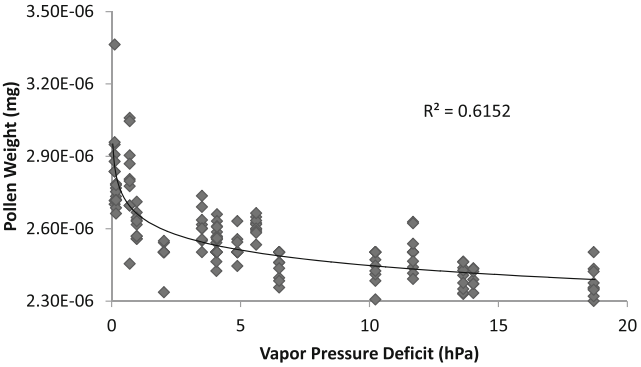

Fig. 1 Average weight per pollen grain of *J. ashei* at 2-h reading plotted against VPD with logarithmic fit line ($p<0.0001$) for temperature (20, 15, and 4 °C)×humidity (20, 40, 50, 76, 85, 97 %) combinations

Juniperus ashei

At 20 °C, *J. ashei* pollen weight was significantly affected by humidity level ($F_{1,174}=55.9$, $p<0.0001$), slide treatment ($F_{2,174}=8.19$, $p<0.0005$), and the humidity×slide treatment interaction ($F_{2,174}=76.1$, $p<0.0001$). As expected, the slide treatment and the slide treatment×humidity interaction were significant factors affecting weight gain. The control slides' treatments lacked pollen; thus, there was no weight gain due to water absorption. Incubation time was not a significant factor affecting pollen weight ($F_{1,174}=0.09$, $p=0.77$) nor were any of the other interaction factors (time×humidity $F_{1,174}=1.32$, $p=0.25$; time×slide treatment: $F_{2,174}=0.77$, $p=0.46$; time×humidity×slide treatment: $F_{2,174}=0.28$, $p=0.76$).

J. ashei pollen weight was significantly affected by humidity level at 15 °C ($F_{1,113}=64.17$, $p<0.0001$). Additionally, weight was affected by slide treatment and the interaction humidity×slide treatment (respectively, $F_{2,113}=5.81$, $p<0.005$ and $F_{2,113}=114.62$, $p<0.0001$). Time and the remaining interaction effects were not significant factors (time: $F_{1,113}=0.12$, $p=0.73$; time×humidity $F_{1,113}=0.66$, $p=0.42$; time×slide treatment: $F_{2,113}=0.41$, $p=0.67$; time×humidity×slide treatment: $F_{2,113}=0.66$, $p=0.52$). The pollen-dusted

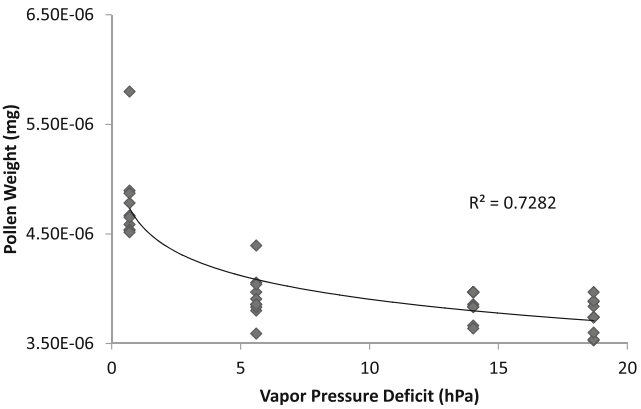

Fig. 2 Average weight per pollen grain of *J. pinchotii* at 20 °C and the 2-h reading plotted against VPD with logarithmic fit line ($p<0.0001$)

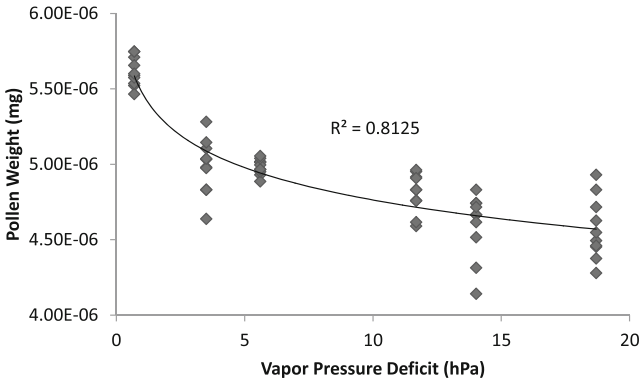

Fig. 3 Average weight per pollen grain of *J. monsperma* at 20 °C and the 2-h reading plotted against VPD with logarithmic fit line ($p<0.0001$)

slide weight fluctuated with increased humidity which is why the slide treatment and the interaction humidity×slide treatment were significant.

For the *J. ashei* 4 °C experiment, humidity and the interaction humidity×slide treatment were not significant factors affecting weight change (respectively, $F_{1,173}=0.72$, $p=0.40$ and $F_{2,173}=0.58$, $p=0.56$) but the interactions time×humidity, time×slide treatment, and time×humidity×slide treatment were significant (respectively, $F_{1,173}=10.59$, $p<0.005$, $F_{2,173}=9.18$, $p<0.0005$, and $F_{2,173}=4.02$, $p<0.05$). The weight of pollen-dusted slides in the 4 °C 97 % humidity treatment continued to increase with time causing the abovementioned interactions that involved time. Slide treatment and time were not significant factors (respectively, $F_{2,173}=1.01$, $p=0.37$ and $F_{1,173}=2.02$, $p=0.16$).

The change in weight for *J. ashei* pollen across the range of humidity treatments was similar for all three temperature levels. Estimated average weights after 2 h for the 97 % humidity treatment at 4, 15, and 20 °C were, respectively, 2.77×10^{-6} mg, 2.76×10^{-6} mg, and 2.79×10^{-6} (Fig. 1). However, the 6-h mean weight for the 4 °C, 97 % humidity treatment was 2.91×10^{-6} mg while the 97 % humidity treatments for 15 and 20 °C only deviated slightly from 2-h readings. In spite of the deviation at the 6-h reading for the 4 °C experiment, the two-way repeated measures ANOVA for the three temperature treatments for *J. ashei* showed that temperature did not have a significant effect on pollen weight ($F_{1,476}=0.23$, $p=0.63$).

In order to examine weight change across the range of humidity levels and temperatures, vapor pressure deficit (VPD) values were calculated (Table 2) and a regression analysis was used to test the relationship between pollen weight and VPD (Fonseca and Westgate 2005). A logarithmic fit yielded an R^2 value of 0.66 ($p<0.0001$) for the 2-h weights (Fig. 1). Since time was not a significant factor affecting *J. ashei* pollen weight, only 2-h readings are plotted (logarithmic fit for 6-h weights $R^2=0.62$, $p<0.0001$).

Table 2 Vapor pressure deficit values (hPa) for temperature and relative humidity combinations

Temp/RH	20 °C	15 °C	4 °C
20 %	18.70	13.64	6.50
40 %	14.03	10.23	4.88
50 %	11.69	8.53	4.07
76 %	5.61	4.09	2.03
85 %	3.51	2.56	0.98
97 %	0.70	0.17	0.12

Juniperus pinchotii

Humidity, slide treatment, and the interaction humidity×slide treatment were significant factors affecting weight change for *J.pinchotii* (respectively, $F_{1,114}=71.5$, $p<0.0001$, $F_{2,114}=3.8$, $p<0.05$, and $F_{2,114}=43.5$, $p<0.0001$). Time and the interaction time×slide treatment also significantly affected weight ($F_{1,114}=4.7$, $p<0.05$ and $F_{2,114}=4.1$, $p<0.05$), but the remaining interaction effects were not significant factors (time×humidity: $F_{1,114}=0.08$, $p=0.78$; time×slide treatment×humidity: $F_{2,114}=0.46$, $p=0.63$). As mentioned previously, the humidity×slide treatment interaction is an expected result because the control slides should not be affected by humidity and pollen gains weight due to water absorption. The time and time interaction effect was in part due to weight fluctuation at the different time intervals for the pollen-dusted slides at the 76 and 97 % humidity treatments. The 2-h mean weight was slightly heavier than the 6-h weight for the 97 % humidity treatment and vice versa for the 76 % humidity treatment. A simple regression using weight and VPD shows a positive, significant correlation between humidity and pollen weight for the 2-h time interval (Fig. 2) as well as the 6-h time interval (not shown $p<0.0001$, $R^2=0.73$).

Juniperus monosperma

For *J. monosperma*, the slide treatment×humidity interaction was the only significant factor affecting weight change ($F_{2,174}=4.0$, $p<0.05$). Humidity, slide treatment, time, and the interactions, time×humidity, time×slide treatment, and time×humidity×slide treatment were not significant factors affecting weight change (respectively, $F_{1,174}=0.002$, $p=0.96$, $F_{2,174}=1.78$, $p=0.17$, $F_{1,174}=0.21$, $p=0.65$, $F_{2,174}=0.92$, $p=0.40$, $F_{1,174}=0.88$, $p=0.35$and $F_{2,174}=0.75$, $p=0.47$). The major effect was that the weight of the pollen-dusted slides increased substantially more than control slides across the range of relative humidity levels. Simple regression using pollen weight and VPD shows a positive, significant correlation between humidity and pollen weight for the 2-h time

interval (Fig. 3) as well as the 6-h time interval (not shown $p<0.0001$, $R^2=0.77$).

Diameter/settling rate

Mean diameter of *J. ashei* pollen exposed to 20 % RH and 20 °C was 18.51 μm, and the mean diameter for pollen exposed to 97 % RH and 4 °C was 19.36 μm (Table 3). For *J. monosperma*, mean diameter was 23.9 for 20 % RH and 24.1 μm for 97 % RH. *J. pinchotii* diameters were 21.2 for 20 % RH and 21.6 μm for 97 % RH. Changes in diameter sizes were not significant for *J. monosperma and J. pinchotii* ($t=0.013$ $p=0.910$ and $t=0.005$ $p=0.944$, respectively). The two-way ANOVA was significant for *J. ashei* pollen diameter ($F_{3,424}=10.13$, $p<0.0001$). Humidity and temperature were significant factors affecting *J. ashei* diameter size (respectively, $F_{1,212}=12.07$, $p<0.0006$; $F_{1,212}=17.86$, $p<0.001$). The interaction humidity×temperature was not significant ($F_{1,212}=0.46$, $p=0.498$).

Pollen weight change was not proportional to pollen diameter change from 20 to 97 % RH for any of the three pollen types which meant that density of the pollen grains increased with the increase in humidity. This had an effect on the calculated settling rate (Table 3). Although the change in weight was not proportional to the change in size for any of the species of pollen, the greatest change in settling velocity was correlated with the pollen type with greatest percent change in weight (Table 3).

The calculated settling rates showed varying levels of change across species, temperatures, and humidity. A change in humidity had a greater effect on settling rate than a change in temperature (Table 3). The greatest changes in settling rates for *J. ashei*, *J. monosperma*, and *J. pinchotii*, were 16, 18, and 24 %, respectively (Table 3).

Discussion

The estimated average weight per pollen grain for *J. ashei* at 60 % RH was 2.5×10^{-6} mg in this study, but Pettyjohn and Levetin (1997) calculated the average weight of a *J. ashei* pollen grain to be 4.6×10^{-6} mg. The mean diameter of the *J. ashei* pollen in this study was between 18.51 and 19.36 μm depending on temperature and humidity. Wodehouse (1935) reported that dry pollen sizes of *J. ashei* ranged from 18.2 to 21.6 μm in diameter and moist grains ranged from 20.5 to 22.8 μm in diameter. It is possible that the weights from this study differ from that of Pettyjohn and Levetin (1997) due to a difference in mean pollen size. The *J. ashei* pollen used in this study was collected from a single location in central Texas near the town of Lampasas. Pollen observed in some other locations was larger than the Lampasas pollen (data not shown). It is not clear whether sizes vary widely from cone to cone, tree to tree, or location to location.

Response to relative humidity at the chosen time intervals was relatively uniform. In most cases, virtually all of the size and weight change happened in the first 2 h. These changes may have occurred much faster than 2 h. Unfortunately, since it can take several minutes for a humidity chamber to equilibrate, shorter time intervals were not possible, especially for lower relative humidity treatments. This means that actual response time cannot be measured. Nevertheless, the experiment shows the range in possible changes. Pollen weight change response time due to changing relative humidity remains to be tested on anemophilous pollen.

One result that may represent a deviation from the response of a related species is the lack of exine shed. Although *C. arizonica* pollen grains shed their exines at 100 % relative humidity between 6 and 24 h of exposure (Chichiricco et al. 2009), exine shed in this study was negligible. This study used 97 % relative humidity and 6 h was the maximum time for

Table 3 Density and viscosity of air and settling rate of *J. ashei*, *J. monosperma*, *and J. pinchotii* pollen at two temperatures and two levels of relative humidity

Temp °C/RH%	VPD (hPa)	Density of air g/cm^3	Viscosity of air g/cm s	Diameter μm (SD)	Weight ng (SD)	Density g/cm^3	Settling rate cm/s
J. ashei							
20/97	0.70	1.198×10^{-3}	1.85×10^{-4}	18.97 (2.26)	2.87 (0.18)	0.79	0.83
20/20	18.70	1.204×10^{-3}	1.83×10^{-4}	18.51 (2.50)	2.37 (0.07)	0.72	0.73
4/97	0.12	1.271×10^{-3}	1.76×10^{-4}	19.36 (2.03)	2.91 (0.19)	0.77	0.88
4/20	6.50	1.274×10^{-3}	1.75×10^{-4}	19.06 (2.07)	2.45 (0.06)	0.68	0.76
J. monosperma							
20/97	0.70	1.198×10^{-3}	1.85×10^{-4}	24.08 (2.63)	5.55 (0.10)	0.76	1.29
20/20	18.70	1.204×10^{-3}	1.83×10^{-4}	23.94 (2.77)	4.58 (0.17)	0.64	1.09
J. pinchotii							
20/97	0.70	1.198×10^{-3}	1.85×10^{-4}	21.59 (2.98)	4.63 (0.29)	0.88	1.24
20/20	18.70	1.204×10^{-3}	1.83×10^{-4}	21.61 (3.22)	3.78 (0.12)	0.72	1.00

exposure. It is not clear whether the reason for the discrepancy in exine shed is due to differences in species or time of exposure. Since Lubriseal is a non-water-based substance and the pollen was resting on the surface of the grease, it is not anticipated that the Lubriseal had any effect on the hygroscopic experiment or exine shedding. It is possible that 100 % relative humidity treatments by Chichiricco resulted in water droplets caused by condensation—which would mean that the pollen grains were suspended in water—resulting in the dissolution of the operculum. The *J. ashei* pollen grains used in this study were observed suspended in solutions of different water activity levels (data not shown), and exines were often rapidly shed in these conditions.

Percent weight change for the juniper species was in a similar range to that of *Salix*, *Betula*, and *Narcissus* as reported by Pope (2010). The greatest changes in weight for *J. ashei*, *J. monosperma*, and *J. pinchotii*, were 25, 21, and 24 %, respectively, while Pope reported average increase in mass due to water uptake at 75 % humidity to be 16 % (Pope 2010). Unfortunately, since Pope's reported range was from 0 to 75 %, a direct comparison cannot be made (Pope 2010).

Durham (1946) estimated the settling rate of pollen by timing its travel through a 1-m tube. He found the rate of fall for *J. ashei* to be 1.16 cm/s which is faster than the fastest *J. ashei*-calculated speed from this study. However, Durham (1946) reported that the mean diameter of the *J. ashei* was 22.8 μm. This is closer to *J. pinchotii* and *J. monosperma* diameters, and the 1.16 cm/s rate does fit within their range of settling rates (Table 3). Also, comparisons of observed settling rates to calculated settling rates have often been found to be quite different. Durham (1946) suggested that the design, specifically the diameter, of the chamber used to observe the fall of pollen can affect the speed of fall. Also, static charge on pollen grains can create large clumps of pollen which fall faster than individual grains creating a downdraft and thus increasing the rate of fall in individual pollen grains but Durham (1946) tried to remove static charge.

A common scenario in the *J. ashei* pollination period would be daytime temperatures around 20 °C with 20 % RH and nighttime temperatures around 4 °C with very high relative humidity. This scenario does produce differences in settling rates; however, the differences are minimal compared to the expected fluctuations in vertical wind speed. The fastest settling rate was that of *J. pinchotii* with a rate of 1.24 cm/s. At that speed, and in the absence of vertical winds, it would still take about an hour for pollen suspended 50 m above the earth to reach the ground. Although the settling rate of pollen can change dramatically with increase in humidity, the system that transports pollen is complex and it is difficult to make broad statements about how weight gain would affect pollen transport. The intent of this study was to evaluate the magnitude of the effect of changing relative humidity on pollen weight, size, and settling rate. Raw data were intended to serve as inputs for a NASA-funded juniper pollen forecast system. This study was not intended to predict actual or relative pollen transport distances without the aid of dispersion modeling.

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