DNA FROM HERBARIUM SPECIMENS: II. CORRELATION OF DNA DEGRADATION WITH HUMIDITY

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ABSTRACT

Comparisons were made of DNA extracted from leaves of *Juniperus virginiana* stored in 100, 75, 70, 54 and 23% humidity at 21ºC for up to 12 months. Fungal growth was observed in the 100, 75 and 70% humidity tests that resulted in the degradation of the juniper DNA. The DNA in leaves stored at 54.4 and 23.1% RH show no evidence of degradation after 12 months storage. It appears that storage of herbarium specimens at sub-ambient temperatures and at RH less than 55% should be effective in preserving DNA *in situ*. *Phytologia 92(3): 351-359 (December 1, 2010).*

KEY WORDS: DNA, herbarium specimens, humidity, degradation, *Juniperus*.

Recently, Adams and Sharma (2010) reported on DNA extracted from *Juniperus* herbarium specimens ranging from 1 to 80 years old. They found the size of DNA declined with age, but varied considerably for specimens less than 20 yrs. old. After about 20 yr. the size of the DNA appeared to asymptote at about 200 - 500 bp. They concluded that variation in the quality of DNA from recent specimens may be due to drying methods and storage conditions (humidity, temperature).

It is now standard procedure to collect specimens and put some leaves in silica gel for subsequent DNA extraction. Liston et al. (1990) published the first paper that utilized silica gel in the field, although Doyle and Doyle (1987) suggested that drying appeared to be effective in preserving DNA. Pyle and Adams (1989) reported that spinach that was desiccated in Drierite® (anhydrous $CaSO_4$), then

stored under ambient herbarium conditions yielded excellent DNA for up to 2 months, but their next (5 mo.) sample displayed some DNA degradation. Liston et al. (1990) reported that spinach stored in silica gel at 21ºC showed very little degradation after 5 months.

Telle and Thines (2008) reviewed the extraction of DNA and amplification of cox2 from herbarium specimens. They note that despite success in the utilization of animal remains and even coprolites from the Miocene, it is still a major challenge to obtain DNA from many herbarium specimens. They attribute this to suboptimal drying and storage conditions. Telle and Thines (2008) reported large differences in the efficiency of different extraction methods and various DNA polymerases used to amplify cox2.

The maintenance of constant humidity in laboratory chambers can be easily achieved by the use of saturated salt solutions (Young, 1967; Greenspan, 1977). Table 1 shows a number of salts that give a useful range of humidities.

Table 1. Saturated salt solutions useful for maintaining a certain level of humidity (values at 20ºC). Adapted from Young (1967) and Greenspan (1977). Salts used in this study are in boldface.

salt	% humidity	cost/g	sat. soln. g/100ml	$/100ml	rating
lithium bromide	6.61	$1.60	160	$256	*
zinc bromide	7.94	4.46	446	1989	--
lithium chloride	11.3	0.84	83.5	70	**
lithium Iodide	18.56	2.70	165	445	-
potassium acetate	**23.11**	**0.05**	**256**	**12**	***
magnesium chloride	33.07	0.015	54.6	0.8	***
potassium carbonate	43.16	0.10	111	11	***
magnesium nitrate	**54.38**	**0.13**	**69.5**	**9**	***
potassium iodide	**69.90**	**0.19**	**144**	**27**	**
sodium chloride	**75.47**	**0.01**	**35**	**0.4**	***
water	**100.0**	**nil**	**--**	**--**	***

The purpose of this study was to examine the effects of humidity on the stability of DNA in *Juniperus virginiana* leaves to gain a better understanding about the degradation of DNA in herbarium specimens.

MATERIALS AND METHODS

Plant specimen utilized: *Juniperus virginiana* L., *Adams 12286,* cultivated, Gruver, TX. Specimen deposited at BAYLU.

Fresh leaves of *J. virginiana* were air dried for 24 h in a plant press at 40ºC. Then the leaves were thoroughly mixed and a random sample of 5 g of leaves was placed in an aluminum weighing dish and thence into a plastic container that was then sealed (Fig. 1). The hygrometer inside the jar was used to monitor humidity. The excess salt can be seen in the saturated solution the bottom of the jar (Fig. 1).

Figure 1. Sealed container with *J. virginiana* leaves suspended above saturated salt solution.

After intervals of 1, 2, 3, 4, 6, 9, and 12 months, leaves were removed for analyses.

DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions. Genomic DNA was visualized by agarose gel electrophoresis by mixing 4 µl DNA extract plus 1 µl loading buffer and run on a 1.5% agarose gel, at 70 v for 55 min. The DNA size marker consisted of 3 µl pGEM markers and 3 µl λ\HindIII, with 6 µl loaded onto the gel. The images were captured on a Kodak Gel Logic 100

Imaging System, and profile analysis was used to determine the modal DNA size and range of DNA sizes. The DNA from some samples was subjected to PCR amplification. ITS (nrDNA) amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS primers utilized.

RESULTS

In general, there was a rapid decline in the genomic DNA in the 100% RH test with a more gradual decline in the 75% and 69.6% RH tests (Fig. 2). At 2 mos. storage there are noticeable breakdown products in the 100% RH test and some loss of the genomic DNA in the 75% RH chamber (Fig. 2). At 6 mos., much of the genomic DNA is

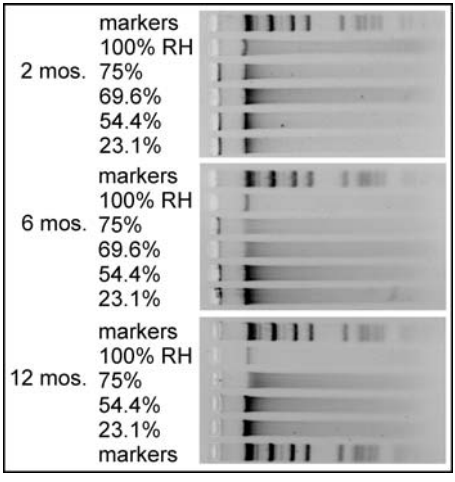

Figure 2. Gels of DNA from 2, 6, and 12 months storage at various humidities. The size marker lanes are λ/HindIII + pGEM.

degraded in the 75% and 69.6% RH tests. After 12 months, only the DNA from 54.4 and 23.1% RH tests appears intact.

Within a few days, a filamentous fungus appeared on the leaves in the 100% RH chamber (Fig. 3). The fungus continued to grow for the next several months. Due to the growth of the fungus, the DNA from the 100% RH chamber maintained a sharp 'genomic' band of fungal DNA (Fig. 2). The DNA from the 100% RH was prepped and the nrDNA region sequenced. A BLAST search in GenBank gave the highest match (67%) to an endophytic fungus in lichen (HM123443). It seems likely that juniper has an endophytic fungus that is not killed by leaf drying. The fungus started to grow in a few days in the 100% RH chamber (Fig. 3).

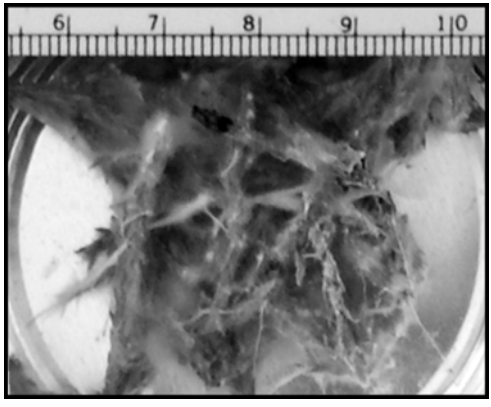

Figure 3. Juniper leaves covered by a white filamentous fungus in 100% RH chamber.

Profile analyses of the DNA after leaf storage for 2 months reveals the breakdown in the 100% RH test and some breakdown in the 75% RH test (Fig. 4). Storage at 69.9, 54.4, and 23.1% RH show very little breakdown after 2 months.

Profile analyses of the DNA after leaf storage for 12 months shows extensive breakdown in the 100 and 75% RH (Fig. 5). Note the sharp genomic peak in the 100% RH test. This is the fungal genomic DNA, as the juniper genomic DNA has been degraded by this time. The genomic peak in the 75% RH test (Fig. 6) is likely fungal DNA as fungal growth was also detected in this test.

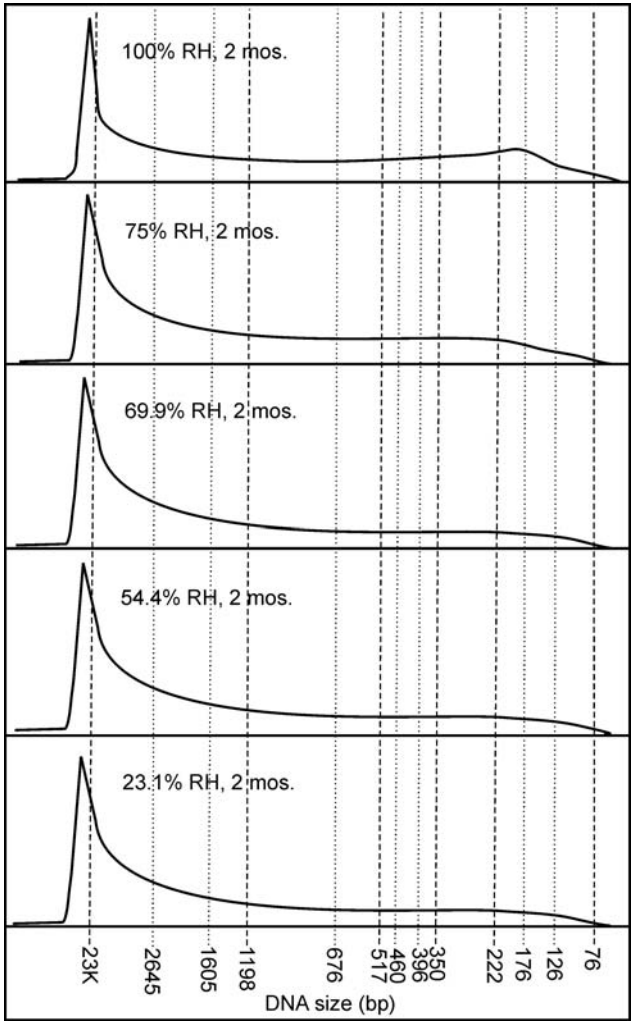

Figure 4. Profile analysis of *J. virginiana* DNA from leaves stored for 2 months at various humidity levels.

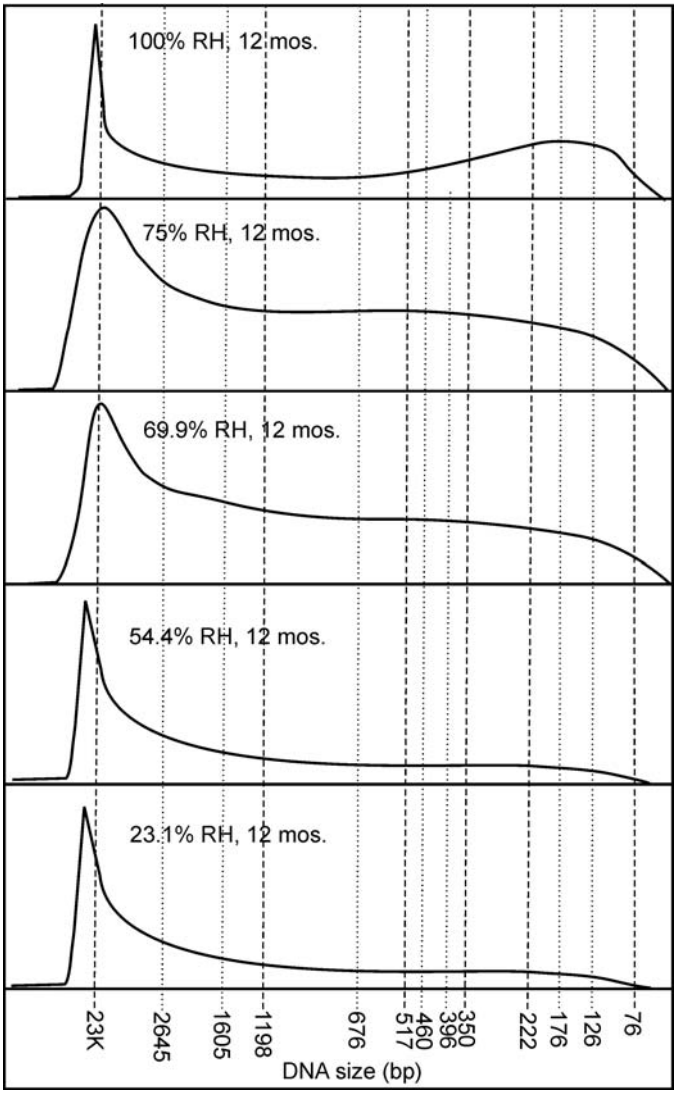

Figure 5. Profile analysis of *J. virginiana* DNA from leaves stored for 12 months at various humidity levels.

The leaves stored at 75 and 69.9% RH are showing some degradation after 12 mos. at RT. Notice the shift of the modal peak to about 23 Kb bp and the more rounded nature of the modal peak (Fig. 5) as well as the long tailing of degraded DNA from 23 Kb down to 76 bp. However, the leaves stored at 54.4 and 23.1% RH yielded genomic DNA with very little breakdown from 2 mo. (Fig. 2) to 12 mo. storage (Fig. 12).

It appears, in this study, that the growth of fungus was the main cause in the degradation of juniper DNA. Fungal growth appears to be inhibited at RH of ~55% or less. Viitanen (1994) reported that the growth of fungi on wood was inhibited at RH less than 80%. They also found that at lower temperatures (eg., 5ºC), fungi would only grow at higher RH (eg. 87 - 90 % RH). Nielsen et al. (2004) found that the lower limit for fungal growth on wood and starch-containing composites was 78% RH at 20-25ºC and greater than 90% RH at 5ºC.

Block (1953) compared mold growth on a variety of substrates (leather, cotton, wood, wool, cheese and glass wool) at various relative humidities at 85ºF (29.4ºC). He concluded that these materials could be stored free of mildew at 65% RH. However, for the internal fungi found in plants, it seems likely that internal fungi are more robust than mildew.

Our herbarium (BAYLU) is maintained at 60ºF and 40% RH to minimize fungi and insect growth. It appears that herbarium specimens stored at ~55% relative humidity should inhibit fungal growth and maintain well-preserved DNA. Unfortunately, many (most?) specimens in herbaria have been exposed to much higher humidity levels before air conditioning was widely utilized.

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