Journal of the Arkansas Academy of Science

Volume 69 Article 9

2015

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Running title: Investigating the Effect of Stratospheric Radiation on Seed Germination and Growth

Abstract

Three seed types: bean (*Phaseolus vulgaris*), corn (*Zea mays*) and radish (*Raphanus sativus*) were flown in a high altitude weather balloon into the mid-stratosphere to investigate the effects of high altitude radiation on germination success and seedling growth. After recovering and planting the seeds, the bean seeds showed lower germination success with exposure to high altitude radiation, and consequently stunted seedling growth. Corn and radish seeds experienced a statistically significant positive effect on germination success from radiation exposure compared to control seeds, but negative effect on seedling growth. Overall, the field experiments presented here support laboratory studies that show radiation exposure on vegetable seeds has a mixed effect on the germination success and negative effect on seedling growth on investigated seed types.

Introduction

With the advent of climate change and the variation in the ozone layer thickness, it is expected that more harmful incoming radiation will reach the lower tropospheric regions. The variation in the ozone layer has been influenced by many factors including anthropogenic activities such as the use of chlorofluorocarbons (CFCs), which have a destructive effect on the ozone layer. The amount of radiation, specifically ultraviolet (UV) reaching the surface depends greatly on the strength of the ozone layer (Krupa 2000). Radiation greatly impacts several physiological and biochemical process in animals and plants (Solomon 1999). Higher radiation levels have been shown to lead to skin damage, generalized DNA damage, eyesight loss in humans, inhibition of cress seedlings and limitations on the anthrocyanin formation in corn (Madronich et al. 2011). Exposure to radiation (specifically, UV) has also been shown to interfere with protein synthesis processes, water exchange, enzyme activity, and leaf-gas exchange (Stoeva and Bineva 2001) in plants. For example, percent germination in seeds and growth rates of seedlings were found to be inversely related to radiation doses in kabuli chickpea plants (Hameed et al. 2008), rice (Maity et al 2005) and in corn and sunflower (Mark and Tevini 1996)

Laboratory research has already investigated the effect of solar irradiation on plants, by exposing the plants to UV in growth chambers (Hollósy 2002). UV-B radiation artificially supplied via filtered lamps into growth chamber was found to impact photosystems I and II, carboxylating enzymes, stomatal resistance, chlorophyll concentrations, soluble leaf proteins, lipids, and carbohydrate pools (Teramura 1983, Hu et al. 2013) While important data have been collected by these studies, these experiments were conducted indoors, creating unrealistic conditions that may exaggerate the influence of radiation on plant processes. This creates a need for field experiments, where the seeds are exposed to radiation in the atmosphere, and their processes investigated to identify what effect the radiation has on plant growth. In this study, we expose three seed types: garden bean (*Phaseolus vulgaris*), corn (*Zea mays*) and radish (*Raphanus sativus*) to stratospheric radiation by using high altitude weather balloons and use germination success and stem growth as indicators to study the effect of radiation on seeds.

Materials and Methods

A 1200 gram latex atmospheric weather balloon was used to lift payload boxes containing high altitude experiments and atmospheric monitoring instruments into the lower stratosphere. The balloon was filled with helium and calibrated to ascend 370 m/min (1200 ft/min) up to 26 km (100,000 ft.) with a flight time of around 90-120 minutes. Payload boxes are attached to the balloon through 100 lb. test strength polyester string line that runs through the middle of the payload boxes. At the end of the line, there was an antenna and GPS recorder for balloon communication and tracking. In between the balloon and the antenna were other payload boxes containing atmospheric monitoring instruments (HOBO data collectors and loggers), and the experimental payload box with the seeds for this study. These data collectors and loggers measured properties of the atmosphere (temperature, pressure, water vapor). A radiosonde (model AnaSonde-2G Anasphere Inc.) collected temperature, pressure, and water vapor while Vernier UV sensors (UVA-BTA and UVB-BTA) measured the ultraviolet exposure during balloon flight. Vernier manufactures education grade equipment, so ultraviolet exposure measured is approximate.

The experiment payload box (Fig. 1) was made of a foam-board measuring 10 x 22 x 30cm (3.5 x 9 x 12") and coated with a heavy duty water repellant (Silicone water guard by Sno-Seal, Item#1336) to keep the box dry. Six 24 Multiwell ™ tissue culture plates were mounted at each side of the box, three outside and three inside the box and secured with screws. Seeds were then added into the culture plates and the lids closed securely. Three seed types were used: bean (*Phaseolus vulgaris)*, corn (*Zea mays)* and radish (*Raphanus sativus)*. Each 24 Multiwell ™ tissue culture plates was packed with 60-72 vegetable seeds, with 3-5 seeds per well. The seeds were divided into three groups: outside, inside and control. Outside seeds were placed outside the payload box for full exposure to high altitude radiation. Inside seeds were placed inside the payload box protected from the outside radiation, Control seeds were left in the laboratory, as a control group with exposure to normal lower altitude background radiation levels.

The weather balloon and payload boxes were launched into the mid-stratosphere. As the balloon ascends the pressure difference between the ground and mid-stratosphere causes the balloon to expand until, at high enough altitude, the balloon bursts. The payload boxes then descends in free fall until reaching the lower troposphere. Then a parachute attached under the balloon is able to catch air and slow the payload boxes for a safe landing on the ground. GPS transmissions by radio allowed tracking and retrieval of the payloads.

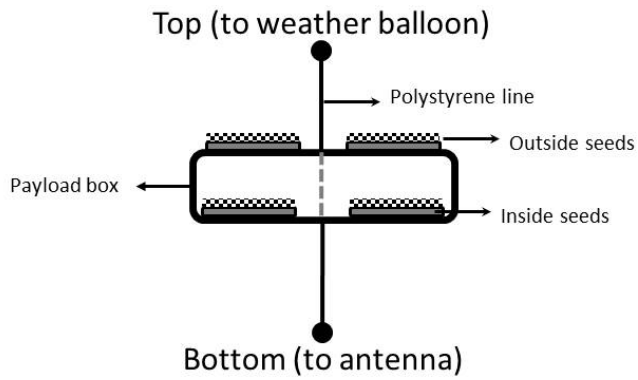

Fig. 1: Experimental payload apparatus showing the different arrangements of seeds in/out of the payload box. The seeds are secured inside multiwell plates, three plates on top, and three inside (only two shown here). Each multiwell plate was covered (checkerboard pattern) with a clear plastic lid.

Under high altitude conditions, outside seeds (outside the payload box) came into contact with low higher levels of radiation (beta and gamma) compared to inside seeds lower levels of radiation because the box shielded gamma and beta radiation. The condition each seed type was exposed to is shown in Table 1.

Table 1: Experimental conditions experienced by the seeds during flight and in the control systems.

Conditions	Seed placement	
	Inside the payload	Outside the payload
Flight time (min.)	90-120	90-120
Max altitude (km)	26	26
Temperature (K) (min-max)	214-326	214-326
Pressure (KPa) (min-max)	0.960-95.0	0.960-95.0
Water vapor content (gm/m^3) (min-max)	4.1-12.4	4.1-12.4
UVA exposure (mW/m^3) (min- max)	0	60-3053*
UVB exposure (mW/m^3) (min-max)	0	14-108*

*approximate exposure as collected with the Vernier, which is an education grade instrumentation

Seeds were removed from the Multiwell plates and planted under 0.25-0.5 inch of soil two days after exposure to high altitude conditions. Varying amount of seeds were planted due to how many seeds were packed into each "well"; 60 corn seeds and 72 bean, and 72 radish seeds were used. Holes were punched into the bottom of germination trays to allow for excess water to drain out of tray. Seeds were placed in a greenhouse and watered every day. Germination success and seedling growth were measured 7 days after planting seeds. Percent germination success was identified by how many seeds germinated from the total seeds planted, while seedling growth was analyzed by measuring the stem length from the soil to the top of the stem.

Statistical analyses were performed in Excel and Kaleidagraph. Briefly, the horizontal bars in the box represent the group median, the box boundaries represent the 25th and 75th percentile values (LQ and UQ respectively) and the "whiskers" in either end represent maximum and minimum values within 1.5 the interquartile distance (IQD), the distance between the upper and lower quartiles (UQ-LQ). Values outside this range are defined by two equations (eq.1-2) where outliers and marked as individual points.

$$\text{height} > UQ + 1.5 \times IQD \qquad \text{eq. 1}$$
$$\text{height} < LQ + 1.5 \times IQD \qquad \text{eq. 2}$$

Results and Discussion

The effect of radiation on seeds was followed by investigating the percent of seeds that germinated. Germination success rates for each type of seeds are shown in Fig. 2. Beans seeds that were in the laboratory (as control) have a germination success of around 88.9%. The seeds that were sheltered (inside the payload box) had a germination succession rate of 93.1%. The seeds that were outside the box, exposed to high radiation showed a germination success of 83.3%.

The germination success for control corn seeds (in the laboratory) was measured at 83.3%. The corn seeds that were inside the box (inside) had a success percentage of 63.8%, a lower success than the control seeds, while the seeds exposed to radiation (outside) have an 86.1%, a slightly higher germination success rate than the control. Radish was also found to increase in germination success upon exposure to radiation. The radish seeds that were outside the payload box showed a 86.1% germination success rate, compared with 77.8% for the control seeds and 76.4% for the seeds inside the payload box. The comparison of germination rates are summarized in Fig. 2.

The total height of control bean plants were found to be statistically different than outside bean plants (p=5.296E-5) and inside bean plants (p=1.768E-3).

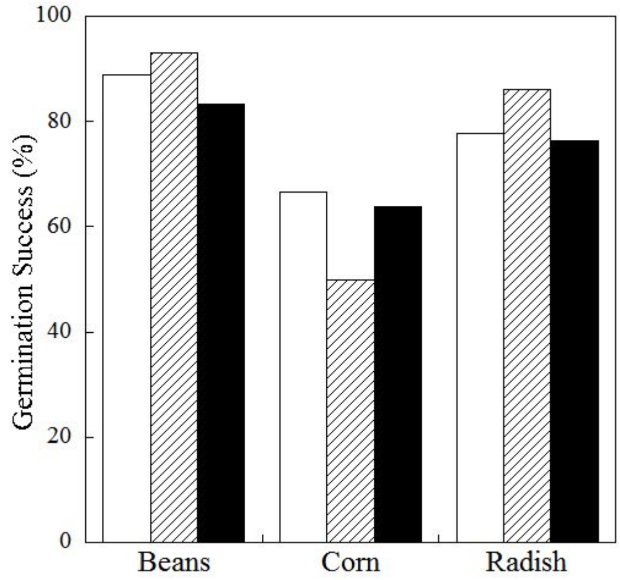

Fig. 2: Germination success with 5% error measured as a percentage of seeds germinated verses seeds planted of a certain type and exposure. The seeds were grouped as control (clear), inside (diagonal), and outside (dark).

Inside bean plants are not statistically different in height compared to outside bean plants (p=2.844E-1).

Bean seedlings have been shown to be sensitive to UVB irradiation and water vapor content. It has been observed that exposure to UVB radiation can halve the fresh weight and leaf area of bean seedlings in comparison to control seedlings that had no UVB irradiation (Tevini, et al. 1981). Rapid desiccation of bean seeds was found to harm seed integrity and impact seedling vitality (Sanhewe and Ellis 1996).

Control radish plant height is not statistically different than outside radish plant height (p=4.688E-1), but is statistically different than inside radish plant height (p=3.956E-2). Inside radish plants are statistically different in height compared to outside radish plants (p=1.721E-5). Control corn plant height is not statistically different than outside corn plant height (p=8.345E-1), but is statistically different than inside corn plant height (p=9.351E-2). Outside corn plant height is not statistically different than inside corn plant height (p=1.068E-1). All p-value calculations are summarized n Table 2.

Seed germination in outside bean seeds (exposed to radiation) was not significantly affected in comparison to the control, with a decrease of around 5.6%. The inside bean seeds showed no effect from the radiation. This indicates exposure to radiation does not significantly affect the germination success of bean seeds.

Table 2: P values comparing seedling height of different group of seeds. P <0.05 indicated with a star show compared groups are statistically different.

Group 1	Group 2	*p*
Control bean	Outside bean	5.296E-5 *
Control bean	Inside bean	1.768E-3 *
Inside bean	Outside bean	2.844E-1
Control corn	Outside corn	8.345E-1
Control corn	Inside corn	9.531E-2 *
Inside corn	Outside corn	1.068E-1
Control radish	Outside radish	4.688E-1
Control radish	Inside radish	3.956E-6 *
Inside radish	Outside radish	1.721E-5 *

The germination success of outside corn seeds show slightly higher germination success compared to control. This increase can be explained by UV promotion. Epigenetic research has shown that UVC radiation affected germination rates due to shifts in methylation patterns of satellite and transcribed DNA (Sokolova et al. 2014). The very low germination success rate of corn inside the box cannot be explained, presently. Outside radish seeds had a higher percent germination compared to control, possibly due to UVA growth promotion. This promotion was previously found to be associated with an increase in chlorophyll content and photosynthetic activity (Tezuka et al. 1994).

When the seedling growth (as measured by stem height from the soil) was investigated, there were no significant changes between the three seed types investigated as seen in Fig 3b. The inside bean seeds at 17 cm show a faster growth rate than the control (13 cm), close to the average height of outside bean seeds which was measured at 18 cm (about 38% higher than the controls) . The average corn plant height for the outside seeds were measured at 7 cm, slightly less than the control seeds (8 cm), while the inside seeds height were significantly lower (6.2 cm), than the controls seeds as seen in Fig. 3b. The improvement on growth rates of seedling height with radiation exposure has also been observed with rice (*Oryza sativa L.*) although, the growth rates were found to decrease upon further radiation exposure (Maity et al. 2005).

Inside radish seeds were statistically significantly shorter than control radish seeds and outside radish seeds (Table 2). The average height of radish plants (2.5 cm) was shorter than the average height of outside and control seeds (3 cm) as seen in Fig 3c. The distribution of the plant height was not affected because the size of the box, which represents 85% of the data set that fits within 95%, is the same height (about 1 cm difference). The outside radish seeds are taller than inside radish seeds because outside seeds are exposed to higher levels UV radiation in addition to cold temperatures. Growth promotion has been shown with UVA exposure to radish seeds, and has been explained by an increase in chlorophyll content and photosynthetic activity from the ability to undergo cellular respiration (Tezuka, et al. 1993). Radish seedlings were not found to be influenced by UVB irradiation (Tevini, et al. 1981). Therefore, the increase in germination success must be influenced by UVA exposure and not UVB exposure.

Plans for future research include measuring UVC profile to quantify the exposure on the seeds. Also, plans to look into the F1 and F2 generations of plants, specifically seedling height and fecundity.

Conclusions

Bean, corn and radish seeds were transported to the mid stratosphere in a payload box carried by a weather balloon and exposed to stratospheric radiation. The seeds were planted in soil to investigate the effect of radiation on their germination and growth. .All seeds types responded to exposure to radiation, with the bean seeds showing a negative effect on germination success but a slight enhancement in seedling growth compared to control. The corn and radish seeds exposed to radiation had a higher germination success when compared to control, but a mixed response compared to control. The field experiments presented here support other laboratory studies on germination and seedling growth, highlighting another important aspect on the response of vegetation to the change in radiation levels due loss of ozone layer resulting from climate change.

Acknowledgements

Funding was provided by Arkansas Science Technology Authority (ASTA) summer internship program, and Arkansas Space Grant Consortium (ASGC). The authors thank Dr. Bob Bennett for his contribution and assistance to experimental design. We would like to acknowledge the various teachers and students involved in the BalloonSAT program.

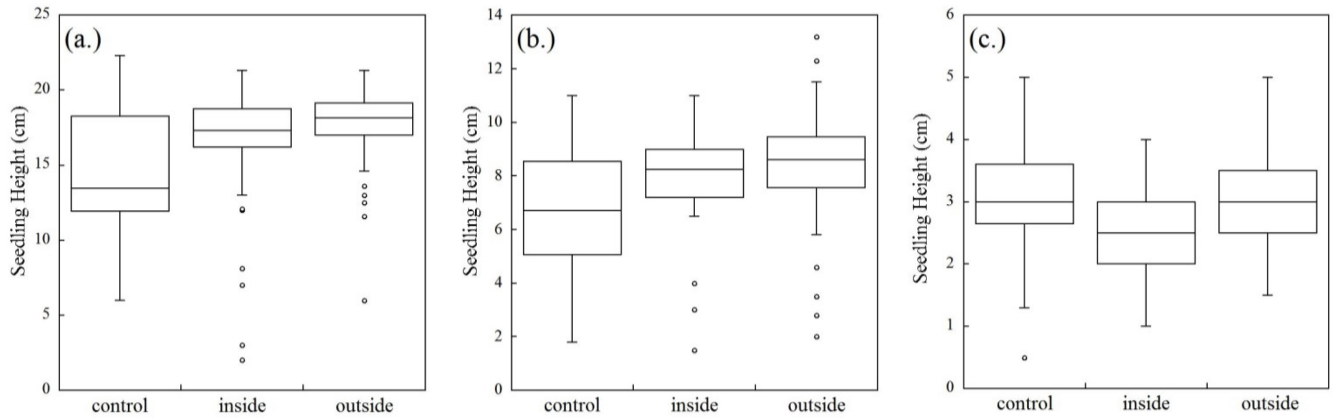

Fig. 3: Box plot of seedling growth of different seeds on a) beans, b) corn, and c) radish. Points represent outliers that are outside the range 1.5x interquartile distance.

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