Cardinal Temperatures and Thermal Time for Seed Germination of Industrial Hemp (*Cannabis sativa L.*) ©[1]

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INTRODUCTION

Hemp (*Cannabis sativa L*.) has been grown for its fiber, oil-rich seed, and psychoactive resins for over 6000 years (Hermann, 2008). Today, it is estimated that over 25,000 different food, fiber, and medicinal products can be derived from the hemp plant (Johnson, 2015). Most modern production of industrial hemp currently occurs in China, Canada and other European countries. Section 7606 of the Agricultural act of 2014 has authorized the production of industrial hemp through cooperation of state Departments of Agriculture, farmers, and university pilot research projects (Kaiser et al., 2015). Prospective industrial hemp farmers in states such as Kentucky will rely on high quality commercial seed in order to support the development and cultivation of a new crop.

Seed germination and dormancy are primarily regulated by moisture and temperature, of which temperature can initiate or inhibit germination of imbibed seeds, as well as control the rate of embryo growth towards germination once germination has been initiated (Bradford, 2002). Seasonal changes in temperature are primary environmental stimuli for seeds to lose primary dormancy. Hemp seeds are considered non-endospermic and non-dormant with a high embryo to seed ratio (Parihar et al., 2014).

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[1]Third Place – Graduate Student Research Paper Competition

Three ‘Cardinal’ temperatures usually describe the broad response of germination to temperature. Germination does not occur below the minimum temperature (T_b), or above a maximum (T_c), while germinating most rapidly in the optimal range (T_o) (Bradford, 2002). According to Bradford, the germination rate of a given seed fraction increases linearly between T_b and T_o when the germination rate (inverse of time to germination of a specific seed fraction) is plotted against temperature. The germination rate decreases linearly between T_o and T_b, but in some cases there is a plateau in germination rate around T_o, as well as variation in T_c among individual seeds in a given population (Bradford, 2002).

The objectives of this study were to investigate seed germination in industrial hemp (*Cannabis sativa* L.) at different constant temperatures to establish base, optimum, and maximum temperatures and to determine thermal time to germination. This information will be useful for establishing industrial hemp production guidelines in more southern latitude’s than that of the Canadian hemp industry which is currently a major source of commercial planting stock.

MATERIALS AND METHODS

Seed source. Seeds of seven hemp (*Cannabis sativa* L.) seed varieties (‘Georgiana’, ‘Tisca’, ‘Finola 15’, ‘Finola 14’, ‘Canda 15’, ‘Canda 14’ and ‘Victoria’) were obtained from the company Hemp Oil Kentucky. Seed was stored in a 10 ˚ C cold-storage room.

Standard Germination. Seeds of hemp (*Cannabis sativa L.*) varieties ‘Georgiana’, ‘Tisca’, ‘Finola 15’, ‘Finola 14’, ‘Canda 15’, ‘Canda 14’ and ‘Victoria’) were placed in plastic petri dishes (100 x 15 mm) and placed in a lighted incubator (8 hours’ light, 16 hours’ dark at approximately 60 µmol sec^{-1} m^{2}) at 20-30°C. Standard germination counts followed the International Seed Testing Association (2008) rules. Four replications of 25 seeds for each seed lot were germinated in Petri dishes on 2 pieces of grade 8001 germination paper (Stults Scientific

Co., Mt. Holly Springs, PA, USA), moistened with 6 ml of deionized water, and sealed with parafilm (Bemis Flexible Packaging, Neenah, WI, USA). Germination was under alternating cycles of 16 hours at 20°C and 8 hours at 30 °C. Standard Germination counts occurred at 7 days, of which three of the high-germinating lots were chosen for subsequent experiments investigating thermal time.

Thermal Time. Germination was measured at temperatures ranging from 3 to 42°C. Two, 25-seed replicates of each of the Georgina, Tisca, and Victoria varieties were planted in Petri dishes in each of six columns spanning the temperature gradient produced by a thermogradient table (Fig. 1). The thermal gradient was produced by pumping chilled water from a recirculating water bath (Polyscience, Niles, IL), and heated water from a Neslab RTE-211 water bath (Neslab Instruments INC., Portsmouth, NH, USA) into opposite ends of an aluminum reservoir plate framed with Plexiglas (Fig. 1). The water baths were connected to four valves on opposite sides of the table with a hose clamp and flexible Tygon R-3606 tubing (Norton Performance Plastic Corporation, Akron, OH, USA). Temperature was measured each time germination data was recorded with a Taylor 1441 E temperature probe (Taylor Precision Products, La Cruces, NM, USA) by placing a row of empty petri dishes filled with water to correspond to each column along the gradient.

Seeds were observed up to five times per day on the first day when germination was rapid, and then gradually reduced to once per day until no additional germination was observed. Seeds were counted once the radicle protruded from the seed coat 2mm. Experiments occurred at 3 temperature ranges due to the limitations of the thermogradient table, but were replicated and repeated.

Viability. A viability test was conducted to distinguish between dormant and non-viable (dead) seeds that did not germinate at 42 °C. 50 Non-germinated seeds were moved from the thermogradient table into a lighted incubator (20-30 °C) for 14 days to determine if germination would resume. After 14 days, seed had not germinated and embryo's where removed from the pericarp and seed coat removed with forceps for staining. The excised embryos were soaked in 0.1% TZ solution (2,3,5 Triphenyl tetrazolium chloride) at 20-30 °C for 18 hours. Another study was conducted following these results by incubating 2 replicates of 25 imbibed 'Georgiana' seeds at 42 °C in an incubator for 24, 48, 72, and 96 hours before staining in a 1% TZ solution. Seeds were evaluated and separated into viable and non-viable seeds. Viable seeds were either totally stained or stained greater than 1/3 of the radicle or cotyledon. Non-viable seeds were either unstained or stained less than 1/3 of the radicle or cotyledon.

Data Analysis. Data collected from petri dishes were used to calculate germination percentage as well as T_{50} values (days to reach 50% germination) at each temperature to describe speed of germination. Germination rate was modeled by plotting the reciprocal of days taken to reach 50% germination against temperature.

RESULTS AND DISCUSSION

Standard germination. Standard germination for the Georgiana, Victoria, and Tisca seed lots were high (greater than 70%), while the other's performed poorly (Table 1). Georgiana was selected for further investigation to allow for more repetitions.

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Cardinal temperatures. Estimates for the low and high cardinal temperatures occur at 3 and 42°C, respectively (Fig. 2). The optimal temperature for germination is between 19 and 23°C, where germination percentage was highest and germination speed was high (Fig. 2). Final germination of 'Georgiana' hemp seeds sown on the thermogradient table was highest (between

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86-88%) at 10 to 23°C and decreased with increasing temperatures above 27°C with a supra-optimal limit of 42°C where no germination occurred. Similarly, in a study by Parihar et al. (2014), there was no significant difference in final hemp seed germination recorded at 20, 25 and 20-30°C. However, in their study germination was not observed at 15 °C and thermo-inhibition occurred at 30 and 35°C. This suggests that commercial hemp seed is a cool season germinator similar to brassica crops. Non-germinating seeds at 42°C were examined for viability using a 0.1% TZ solution and were over 70% viable after 4 days. This suggests that these seeds were either thermally inhibited or induced into secondary thermal dormancy.

Time to 50% germination (T_{50}) decreased with increasing temperature and was lowest at 35 °C, although the T_{50} for 29, 31, 35, and 37 °C were not significantly different. Conversely, germination speed (T_{50}) became slower with increasingly lower temperatures between 19 and 10°C (Fig. 2). The faster germination rate at temperatures above optimum is not typical in seeds of most species where time to 50% germination gets slower at supra-optimal temperatures (Bradford, 2002). However, in some species, germination rate tends to plateau at supra-optimal temperatures (Orozco-Segovia et al., 1996) as observed in 'Georgiana' hemp seeds (Fig. 2).

Thermal time. Plotting the reciprocal time (hours) to reach 50% germination (G_r)against temperature showed a linear relationship of increasing germination rate with increasing temperature between 15 and 29°C (Fig. 2). The regression equation generated from this data provides a putative thermal time model to predict time to 50% germination, where the cumulative Gr at Tx for each hour sums to 1.

Literature Cited

Bradford, K. J. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. Weed Sci. 50:248-260.

Cave, R.L., Birch, C.J., Hammer, G.L., Erwin, J.E. and Johnston, M.E. 2011. Cardinal temperatures and thermal time for seed germination of *Brunonia australis* (Goodeniaceae) and *Calandrinia sp.* (Portulacaceae). HortScience 46: 753-758.

Hermann, A.M., 2008. The effect of plant population density and harvest timing on agronomic fiber yield and quality characteristics of industrial hemp (*Cannabis*), cultivar Alyssa, grown in the Parkland region of Manitoba, Canada. Accessed 18 Dec. 2016. http://mspace.lib.umanitoba.ca/handle/1993/29708?mode=full

ISTA. 2008. International Rules for Seed Testing, Edition 2008. International Seed Testing Association (ISTA), Bassersdorf, CH-Switzerland.

Johnson, R. 2015. Hemp as an Agricultural Commodity. Congressional Research Service report 7-5700, RL32725. https://www.fas.org/sgp/crs/misc/RL32725.pdf

Kaiser. C., Cassady,C. and Ernst, M. 2015. Industrial Hemp Production. Center for Crop Diversification Crop Profile. https://www.uky.edu/Ag/CCD/introsheets/hempproduction.pdf

Orozco-Segovia, A., L. Gonzalez-Zertuche, A. Mendoza, and S. Orozco. 1996. A mathematical model that uses Gaussian distribution to analyze the germination of *Manjreda brachystachya* (Agavaceae) in a thermogradient. Physiol. Plant.98:431-438.

Parihar, S. S., Dadlani, M., Lal, S. K., Tonapi, V. A., Nautiyal, P. C. and Basu, S. 2014. Effect of seed moisture content and storage temperature on seed longevity of hemp (*Cannabis sativa*). Indian J. Agricul. Sci. 84: 1303-9.

Roberts, E. H. 1988. Temperature and seed germination. *In:* S. P. Long and Woodward, F.I., eds. Plants and Temperature. Cambridge, U.K: Society for Experimental Biology. pp. 109-132.

Table 1. Standard germination in seven industrial hemp accessions.

Accession		3-day count		7-day count
Georgiana		65		81
Victoria		61		91
Tisca		63		73
Finola (2014)		12		12
Finola (2015)		55		58
Canda (2014)		12		12
Canda (2015)		25		27

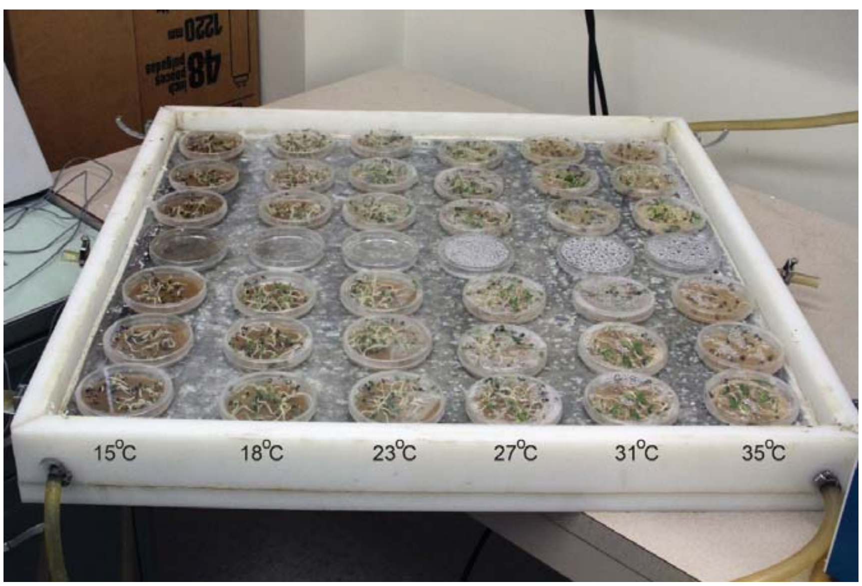

Fig. 1. Thermogradient table.

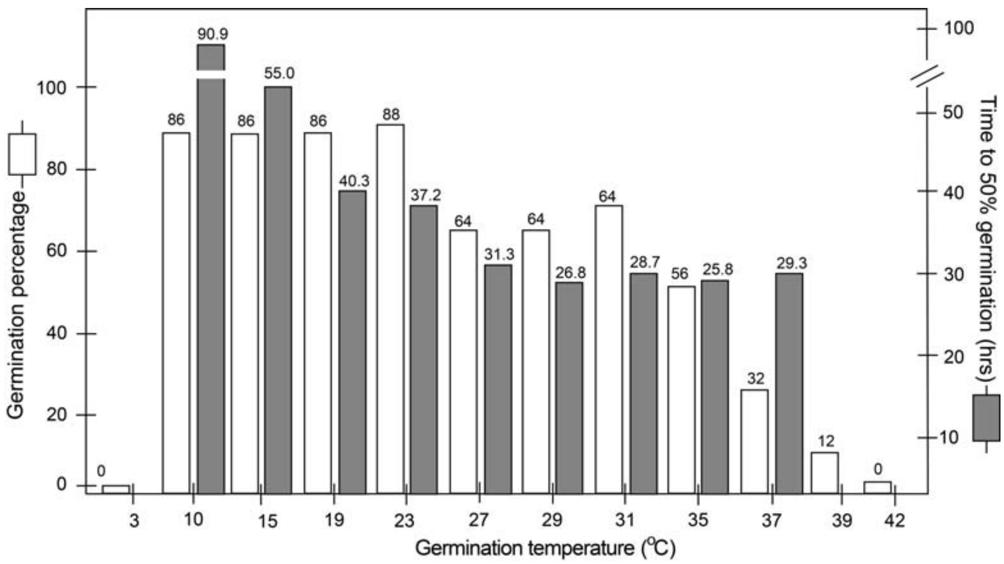

Fig. 2. Germination percentage and speed in ‘Georgiana’ seeds at different temperatures.

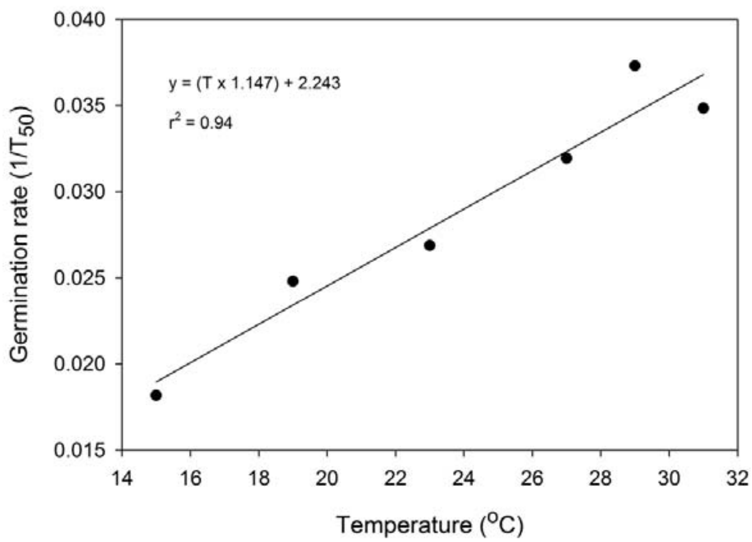

Fig. 3. The relationship between temperature and germination rate in ‘Georgiana’ seeds.