Industrial Hemp (*Cannabis sativa* L.) Germination Temperatures and Herbicide Tolerance Screening

by

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SPES

Industrial hemp (*Cannabis sativa* L.) is a multipurpose crop cultivated for fiber, seed, and flower (pharmacological) outputs. Bast and hurd fibers from hemp stalks can be used in a number of industrial products, including auto parts, textiles and building materials. Hemp seeds can be used as an ingredient in human food and animal feeds, as a source of beneficial oils with unique fatty acid profiles, and as a component of cosmetic products. Industrial hemp is not a commercial crop in Virginia, and information is needed on production and management. Generating information such as suitable temperatures for germination and plant responses to herbicides for varying hemp production systems will be integral to improving productivity of hemp in Virginia. In 2018, industrial hemp cultivars developed across a wide range of latitudes (Canadian, Northern and Southern European) were tested to determine their germination percent and absolute rates at different temperatures. This study was completed on a thermogradient table with temperatures maintained from 0°C to 45°C. No significant differences were observed at base temperatures amongst the varieties. In 2017 and 2018, greenhouse and field studies were conducted to assess herbicide tolerance of industrial hemp. Preemergent and postemergent herbicides were chosen for this study based on their specific mode of action. The greenhouse and field studies indicated that pendimethalin, *S*-metalochlor and fomesafen herbicides appear to be suitable preemergent treatments for industrial hemp production as measured by low phytoxicity and acceptable plant growth. Sethoxydim, bromoxynil, clopyralid, and quizalofop may be suitable postemergents for

industrial hemp production, but some of these treatments did cause some visible injury that was transient in some cases.

General Audience Abstract

Industrial hemp (*Cannabis sativa* L.) has a long history of human use. Early in the 20th century, some predicted hemp would be the first billion dollar crop given its multiple industrial applications. Government policy that restricted, then prohibited, hemp's use in the U.S. prevented that from happening. A reawakening to the versatility and usefulness of hemp for products ranging from engineering fibers and textiles to food and health products has developed over the last 30 years. Hemp-based products are thriving on the market for public demand. In Virginia, passage of legislation in 2017 made hemp a legal cash crop. Appropriate management decisions rely on information available from researchers. However, very few data on hemp production are available for this region. Hemp varieties may differ in part due to the broad range of latitude associated with their source of origin (e.g., from Italy to Finland in Europe) and thus the plant's differential responses to light and temperature regimes. Thus, a factor such as varietal response to soil temperature at germination could be an important variable for successful establishment, which is critical to crop productivity. Stand establishment, in turn, may be affected by factors such as germination temperature, which has implications for planting date.

Along with establishment, few data have been published regarding hemp's tolerance to different herbicides. To date, the only published studies from the Southern region of the United States regarding hemp production in response to herbicide treatments were conducted in Kentucky. Generating basic information on hemp response to temperature for germination and tolerance to herbicides will be important step for developing a suite of useful agronomic practices that support the incorporation of hemp into Virginia cropping systems. The hemp industry's development in Virginia is still in its early stages, and the research described here – focused on questions related to germination temperature and herbicide tolerance – will help to

improve our understanding of and determine suitable agronomic practices for the crop We thus designed experiments to test the following null hypotheses: Industrial hemp will not differ in germination response to temperatures, regardless of source of origin. Industrial hemp will not differ in measures of visible injury, yield, and growth in response to preemergent or postemergent herbicide treatments.

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Chapter 1: Literature Review

Brief Hemp History

Industrial hemp (*C. sativa* L.) has played a major role as a fiber and grain crop for humans through much of our history. Native to Central Asia (Simmonds, 1976; Polio, 2016; Fike, 2016), this historic plant has potential value as a modern cash crop because of its environmental, medicinal, and economic benefits. Hemp's first harvest, probably by the Chinese, dates back 8500 years ago (Schultes, 1970). A member of the Family Cannabaceae, hemp likely originated as a species in higher precipitation regions of Central Asia (Small, 2015), but humanity's long interaction with the *Cannabis* genus and the species' ability to adapt to a variety of edaphic and climatic conditions have led to its near global spread (Johnson, 1999).

Well before the development of agriculture, nomadic peoples likely encountered hemp along rivers where it grew in Central Asia (Small, 2015). Hemp's broad adaptability to varied environments and very different selection pressures (i.e., for fibers or for psychotropic compounds) led to marked regional differences in form and plant chemistry. Human exposure to and interaction with *Cannabis* for much of our history – and selection for these disparate outputs – also led to the development of markedly different attitudes towards the plant. Many of the negative views of the species in more recent times have been related to the potential adverse effects associated with the use of the psychotropic strains. Historically, however, the plant largely was viewed favorably given its high quality fibers.

Industrial hemp's fiber properties have been the basis for its successful spread and use as an agronomic crop. Bast fibers likely have been the most utilized component of the plant historically. The species' bast fibers were likely an early primary source for string for nets and bows and used to make other valuable textile products (Whitford, 1941). Around 1500 BCE, hemp made its arrival to Western Europe from Central Asia (Husbands, 1909). The crop spread throughout the continent and became a vital resource for European maritime countries, whose navies utilized hemp fibers for rope, cordage, and canvas (the word being derived from *Cannabis*; Douglas-Harper Online Etymology Dictionary, 2019). Hemp was a source of power, helping change Europe's national, political, cultural, and economic destiny, and it was in this context that the crop was taken to the New World.

The Spanish government was very encouraged to produce hemp for fiber in the Americas since production in Spain was limited by the country's hotter, drier climate (Clarke and Merlin, 2013). Hemp was imported to South America and cultivation started in what is now Chile. The crop has been grown there for 400 years, largely for local use (Clarke and Merlin, 2013).

In North America, hemp was an important fiber crop from colonial times until the early 20th century (Small and Marcus, 2002). English colonists often were mandated to grow the crop to ensure supplies for the Royal Navy. In the late 1630s, laws in Connecticut, Virginia, and Massachusetts required each family to plant one teaspoon's worth of hemp seed in their yard (Deitch, 2003). Those who did not obey were subject to jail as punishment (Herdon, 1963).

Hemp served as the world's most universal textile fiber until the invention of the cotton (*Gossypium hirsuitum* L.) gin in the American South in the 18th century. Hemp continued to be grown in or imported to the U.S. through the 19th century but was used primarily for low value string and twine – often to bundle up bales of cotton – in addition to its use for rope, rigging, and sails. Movement of ships from sail to steam power reduced demand for hemp, and the fiber also faced competition from other fiber sources (jute and sisal) (Fortenberry and Bennet, 2004).

In the 20th century, concerns about marijuana (the psychotropic strain of *Cannabis*) and potential drug use and abuse led to constraints on industrial hemp production. Passage of the Marihuana Tax Act in 1937 put hemp under regulatory control of the Department of Treasury, and effectively constrained production (USDA, 2000). Government restrictions were eased during World War II, and producers were encouraged to become registered and licensed to grow hemp for the U.S. military (Robinson, 1996), but these prior restrictions were resumed following the war. In 1970, the Controlled Substances Act designated all forms of *Cannabis* as Schedule I drugs (USDA, 2000).

In the 1990s, hemp was legalized in Canada and Western Europe, sparking a resurgence of interest in the U.S. by those wanting to develop an American hemp industry (Fike, 2016). In 2014, the U.S. Farm Bill signed by President Barack Obama, legalized research with industrial hemp. The bill allowed state-sanctioned pilot programs to assess the different characteristics and develop management strategies for the crop. According to the National State Conference of Legislatures (www.ncsl.org), at least 39 states in the U.S. currently are engaged in research related to industrial hemp.

Virginia law officially allowed research to begin in 2015 (although the first crops were not planted until 2016). The state's pilot programs are managed under the oversight of the Virginia Department of Agriculture and Consumer Services (VDACS). Virginia and most other states follow federal law in defining industrial hemp as any *C. sativa* subspecies having 0.3% or lower tetrahydrocannabinol (THC) concentration, although a 1% threshold has been accepted in some states.

Market

Hemp is currently cultivated for grain or fiber in at least 30 countries. Canada grew 36,000 ha of hemp in 2016 (Johnson, 2018), largely for grain, while Europe cultivated 33,000 ha (Carus and Sarmento, 2017) and 4,000 ha of hemp were grown in the U.S. Information about the amount of hemp grown in China, Russia, and Australia in 2016 is not available, but much of the Chinese crop is grown for fiber. In the U.S., the value of hemp products sold was greater than $688 million in 2016, and sales are expected to increase 25 to 50% from the previous years (Johnson, 2018). These products include food, cosmetics, and textiles (whether finished goods or raw materials) imported from other countries (Small and Marcus, 2002).

Among states growing hemp in the U.S., Colorado leads production acres (61%). Kentucky (26%) and Oregon (5%) accounted for most of the remaining production among U.S. states in 2016. While there is growing interest in hemp in Virginia, the state produced only 55 ha of hemp in 2018 (Bronaugh, 2018). Along with questions about establishment and agronomic practices, additional research will be needed to address the challenges of production, processing, and market development before this crop becomes a valuable commodity for the Commonwealth.

Hemp for fiber

Hemp grown for fibers can be separated into the long and short fiber fractions. The long “bast” fibers grow outside the vascular cambium and traverse the plant vertically. The short “hurd” (secondary fibers) that grow into the center of the plant from inside the vascular cambium (Salentijn et al., 2015), increasing in quantity as plants mature.

Bast fibers (primary fibers) have high strength to weight ratios and have been used for various textiles. Along with their historic uses for rope, cordage, and canvas, hemp fibers have been used for clothing and historically were a commonly-used resource for items such as shirts, shoes, pants, and jackets. The modern clothing industry mainly requires high quality bast fibers from hemp in order to be competitive with other fiber materials such as silk, cotton, wool, nylon, and polyester. Because of their more coarse nature, hemp bast fibers are often paired with other fibers such as cotton for use in clothing. In recent years, long fibers have been processed for a number of market products such as fabrics, reinforcement for resins used in door panels, and insulation to name just a few. Currently, the most important market for bast fibers is the automotive industry.

The bast fibers have low lignin concentrations and high concentrations of cellulose. These grow in bundles of pericyclic elementary fibers, approximately 20 to 50 mm in length (Salentijn et al., 2015). Bast fibers are considered the higher quality, higher value fiber fraction. Garcia-Jaldon et al. (1998) estimated that bast fibers contain ~55% cellulose, ~16% hemicellulose, ~18% pectin, and ~4% lignin. Limited lignification, high cellulose content, and low numbers of interactions between pectins and structural components of the cell wall are important features for an appropriate extractable fiber for both paper and textile industries (Salentijn et al, 2015; Mandolino and Carboni, 2004).

Core fibers in hemp stalks are called hurd or shiv. The hurd has greater lignin concentration and lower cellulose present (Van der Weff and Van den Berg, 1995). These short fibers have been used to make hempcrete (a mixture of hemp and Portland cement) for building construction, as a bedding material, and it is being explored as an industrial absorbent. Hemp hurd is also used as a primary source in the specialty pulp sector, and construction industries (Karus and Vogt, 2004). Hemp fibers are rich in cellulose, making them a useful primary source for biodegradable materials (Liu et al., 2015). Fiber quality and quantity are also dependent on the agronomic factors associated with production. Variability in primary and secondary fiber yield and quality remains an important area of investigation. Van der Weff and Van den Berg (1995) explored the quality of Dutch and Hungarian cultivars under two planting densities (10 plants m^{-2}, 90 plants m^{-2}). The authors observed an interaction between plant density and developmental stage which was affected by cultivar maturity and fiber quality. Growing conditions and genetic differences also influence the tissue architecture of hemp stems (Fernandez-Tendero et. al., 2017).

Studies from other countries with existing hemp research programs (e.g., France, Italy and Canada) have helped inform production research in the U.S. For example, research in Bologna, Italy, assessed genotype (monoecious and dioecious), plant density, and harvest timing effects on hemp fiber yield during 2003 and 2004. Precipitation in 2004 was more than double the precipitation in 2003 (215 vs. 96 mm) and supported 25% greater fiber yields. However, hemp grown under drought conditions during the 2003 growing season produced fibers that were finer, higher in quality, and had a higher degree of maturity (Amadduci et al., 2008). The authors suggested that extremely hot and dry weather conditions enhanced flowering and lowered yields. Greater plant populations may increase primary fiber content due to plant elongation (vertical growth) associated with intercrop competition (Cromack, 1998).

Hemp's suitability and sustainability for Virginia cropping systems will depend on the different agronomic management practices required to grow the crop for these end uses. It will be essential to find hemp varieties adapted to Virginia's diverse climatic and edaphic conditions that can be managed with modern-day agronomic inputs and practices. Along with varietal choice, factors such as planting date, planting density, and harvest time have a major bearing on hemp's productivity and quality.

Hemp for grain

While largely in the public consciousness as a fiber crop based on its long history for that use, hemp seed have increasingly been recognized for their oil content and unique fatty acid profile. The seeds are high in omega 3 alpha linoleic and omega 6 alpha linolenic acid concentrations and have a high level of protein – typically above 20% (Schultes, 1970). Natural food stores and cosmetic companies find value in selling hemp seed and derivative products. A rapid increase in hempseed product sales worldwide has gained the attention of American citizens due to the dietary benefits for consumers. However, hempseed shelf life represents a particular challenge, because seed quality can deteriorate over time. This may be a function of the time to market from Europe or Canada to the U.S., which would support an argument for domestic production. Hemp seed must also be competitive with other oilseed crops in the marketplace. Current seed yields and high levels of seed shatter limit crop harvests; a future hemp grain industry could benefit with the development of high yielding cultivars through breeding programs.

Industrial hemp grain varieties are grown as summer annual crops. Grain cultivars can either be monoecious (having male and female flowers on the same plant) or dioecious (having male and female flowers on separate plants) (Small and Marcus, 2002). Whether a variety is monoecious or dioecious will affect row spacing and seeding rate requirements at planting. In general, hemp grown as a grain crop is planted at 22 to 34 kg ha^{-1}. Row spacing is often double the row width used to grow fiber lines. This configuration is thought to give the plants more room for flowering and seed development (Cromack, 1998).

Small seeds with low vigor may make hemp more challengeing to establish than more traditional row crops grown in the U.S., such as corn (*Zea mays* L.), soybean (*Glycine max* L.) or cereal grains. Factors such as soil temperature, soil tillage, seeding depth, and weed control will be discussed in the following section.

Latitudinal Adaptation

Industrial hemp is day-length sensitive, resulting in greater vegetative growth if planted earlier (Roth et al, 2018). As days become shorter, four to five weeks after the summer solstice (June 21), vegetative growth slows and flower development is triggered. Early planting takes advantage of this feature, resulting in taller plants with higher fiber yields. However, this decision does not change the harvest date significantly.

Hemp is grown over a broad range of latitudes. Production in Europe occurs from Finland (>60°N) to Italy (~45°N) and in Asia, fiber crops are grown as far south as Yunnan province (24°N). The broad distribution and adaptation of the crop reflects its adaptation to varying day lengths. Thus, planting date should be adjusted based on the origin of seed variety. In the U.S., planting date recommendations for the North-South transition zone generally fall in early May. Despite much cooler climate, farmers in Ukraine may plant as early as mid-March (A. Kinsel, personal communication). Optimal timing for seed germination and stand success may be a function of the interplay between a variety's source of origin and growing conditions such as soil temperature and climate.

Germination Temperature

Information on industrial hemp establishment is limited. Few quantitative data are available on the effect of temperature on establishment and productivity of industrial hemp. Data associated with production potential and optimum crop management can help develop a model that would complement traditional agronomic programs (Lisson et. al., 2000). Temperature germination research with agronomic plants was conducted in the 1800s, but studies of hemp germination and growth responses to temperature could not be conducted with precision until the invention of the thermometer (Lisson et. al., 2000). In the 1900s, these studies took place to determine how the plants grow better and analyze plant productivity (Edwards, 1932).

Haberlandt (1879, cited by van der Werf et al, 1995) saw no germination of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), or white clover (*Trifolium repens* L.) after incubating for four months on a block of ice. Hemp, rye (*Secale cereal* L., pea (*Pisum sativum* L.), and red clover (*T. pretense* L.) showed signs of germination (<10%) on ice after 45 days (Edwards, 1932). In 1875, Uloth (cited by Edwards, 1932) carried out a five-month germination experiment but seeds were either on or between blocks of ice with or without soil. Uloth did not report the percentages but noted that hemp, wheat, barley, rye, oats and pea had similar germination percentages four months after incubation. Gharderi-Far et al. (2010) studied the germination response of yellow sweet clover (*Melilotus officinalis* L.) and estimated the base, optimum, and ceiling germination temperatures were 0, 18.5, and 34.6 °C. In a recent study, the germination response of hemp cultivar ‘Kompolti’ was tested at different temperatures between 1 and 55 °C (Lisson et. al., 2000). The authors reported that the estimates of optimum and maximum temperature of radical length development for Kompolti were correspondingly 29 and 41 °C. Base temperatures were variable throughout the study, ranging from 1 to 6 °C. van der Werf et al. (1995) reported the base temperature for leaf growth (expansion) was 3 °C and development (leaf appearance) occurred at 1 °C. Seeds of different species will be have different germination characteristics at different temperatures. In 1923, Coffman stated that starchy seeds appear to be unable to resist low temperatures to the same degree as the more oily seeds, without injury and reducing germination percentages. Based on these results, hemp appears to have one of the lowest base germination temperatures among field crops.

Soils and fertility

Although often touted as suitable for all types of soils, industrial hemp is best adapted to well-drained loams with pH ranging from 6 to 7. Heavy clay or compacted soils can slow emergence and development, resulting in lower yields (Roth et al., 2018). Seedlings are very sensitive to wet soils or flooding during the first three weeks or until growth reaches the fourth internode (about 30 cm tall). Water-damage can cause stunted growth or even crop failure (Ehrensing, 1998).

Industrial hemp is less commonly grown in sandy, infertile soils (Roth et al., 2018). Such soils, with low organic matter, limited cation exchange capacity, and poor structure typically are drought-prone and incapable of supporting sufficient plant growth without substantial inputs. Accordingly, high levels of nutrient inputs and irrigation may be required to achieve maximum yields, but in turn may make production uneconomical (Roth et al., 2018).

Fertility recommendations for hemp grain crops vary by soil type, but frequently are considered similar to that for corn (*Zea maize* L.) or wheat production. The crop may require relatively high levels of nitrogen (N). Researchers in Western Canada found seed yield response to N was linear or quadratic at up to 120 kg N ha^{-1}, and the nature of the response differed by cultivar (Vera et al., 2004). Subsequent work by these authors suggested maximum seed yield might occur between 175 and 200 kg N ha^{-1}, although this varied by cultivar. Similar results were reported for Eastern Canada, with grain yield increasing 2.5-fold (1670 vs. 4210 kg ha^{-1}) over the control (0 N) when plots received 200 kg N ha^{-1}. However, results from Europe suggest in other environments hemp may be less responsive to applied N (Tang et al., 2017).

Moderate to high levels of phosphorus (P), and potassium (K) have been recommended also (e.g., see, Kaiser et al., 2015), although little response to P is observed on soils with adequate levels (Vera et al., 2010; Aubin et al., 2015).

Hemp should be planted into a fine, firm seedbed, prepared either with or without tillage (Small and Marcus, 2002). Good seed-to-soil contact is essential for optimum industrial hemp seed germination. The soil can be worked and planted as soon as the ground is dry enough so compaction can be avoided. A shallow, firm seedbed permits seed to be placed at a uniform depth, resulting in a more even seedling emergence. Industrial hemp is normally sown using a standard grain drill and should be planted at a depth of 0.5 to 1 cm (Small and Marcus, 2002).

Seeding rate and timing

Hemp seeding rate, timing and their interaction can have large effects on plant productivity, quality and weed presence. Van der Werf et al. (1995) tested planting at 10, 30, 90, and 270 plants m^{-2} and reported that early season growth rates increased with higher planting density. However, plants died at the highest planting densities due to self-thinning. Although this was associated with lower growth rates, proportions of stem increased with density and stem quality also increased. The authors reported maximum stem yields at about 90 plants m^{-2}. Results from planting density studies conducted in Australia suggest similar maximum yields at 110 plant m^{-2}.

Fiber quality also may be increased with planting density (Khan et al., 2011). Early research in the U.S. suggested that seeding rate could have variable effects on retted straw yields (although seeding rate – 3 to 5 pecks/acre – was poorly defined), probably because high seeding rates were accompanied by high self-thinning (Wilsie et al., 1944). The authors also observed that self-thinning was worse with high N fertility.

When grown for fiber, industrial hemp usually is sown in 15- to 18-cm-wide rows, using every run of the grain drill. Wider (every-other-row) spacing is common for grain production, although research with grain varieties conducted in Canada found no crop production differences between 18 and 36 cm (Vera et al., 2006). Early seeding (after the last frost in the spring) should be considered to support greater weed control and minimize resource competition (Roth et al, 2018). Grain varieties can be planted at a lower rate and with wider row spacing to allow for more branch growth to occur. This rate could be higher if germination is low or if seeds are large in size.

Herbicidal Treatment Options

Weed presence in fields both decreases crop yield and lowers crop quality. Some evidence suggests that industrial hemp can outcompete weeds in field settings given its fast growth, thick foliage, and capacity for rapid canopy closure (Poisa and Adamovic, 2010;

Rehman et al., 2013). Crop density is an important factor in weed suppression. For example, increasing planting density from 100 to 200 hemp plants m^{-2} decreased aboveground weed biomass at the time of harvest by 80% (Hall et al., 2014). Canadian researchers also found reduced weed density with increased hemp planting rates, and differences between cultivars also were observed (Vera et al., 2006).

Although high planting density may be useful for minimizing weed pressure, it will likely not be sufficient in all situations. Weeds are more likely to be a concern in grain systems that rely on lower seeding rates and wider row spacing (Hall et al., 2014) . In such cases, herbicide application (or tillage) may be needed to control weed populations. Few data are available on herbicides suitable for hemp (described below), largely because past restrictions on growing the plant have prevented testing and labeling.

Information is needed on the phytotoxicity of herbicides for hemp production systems. Mode of action of an herbicide refers to the manner in which it affects the biochemistry and overall physiology of plants (Ashton and Crafts, 1973). In turn, the phytotoxicity of an herbicide results from the biochemical, physiological, and consequent changes induced by the chemical. The effectiveness of an herbicide also is a function of its mode of uptake, as well as its fate (location) and method of degradation within the plant (Ashton and Crafts, 1973).

Several factors affect whether herbicides can safely be applied to crops. Some herbicides are selective between grasses and broadleaves, which has implications for the use in a broadleaf crop (Shaner, 2014). Weather also is a factor, as crop seedlings often struggle to metabolize herbicides in wet conditions (Taylor-Lovell et al., 2001), and dry conditions may limit the uptake of some herbicides. Sufficient planting depth and good seed to soil contact are important for shallow-seeded plantings in which preemergent herbicides will be used, as poor planting could allow contact with germinating seeds (Kandel et al. 2018).

Recent research in Kentucky suggests some variation in hemp response to pre- and post-emergent herbicides (Maxwell, 2016). Several herbicides with different modes of action were tested for their phytotoxicity to hemp. Pre-emerge herbicides were applied at the time of planting and post-emergent herbicides were applied 22 to 24 days after emergence at two sites. Mesotrione and trifloxysulfuron were unsuitable for use with hemp as they caused >78% injury to hemp plants; bromoxynil, pendimethalin, and MSMA were considered excellent candidates for weed control in hemp, as they caused little (< 10%) injury (Maxwell, 2016). However, a wide range of herbicides remains to be tested for suitability with hemp production. To date, only Edge® Granular Herbicide (ethalfluralin), a nonselective herbicide has had hemp added to its label, and only for certain provinces in Canada.

Objectives

Generating basic information on hemp response to temperature for germination and tolerance to herbicides will be important steps for developing a suite of useful agronomic practices that support the incorporation of hemp into Virginia cropping systems. The hemp industry's development in Virginia is still in its early stages, and the research described here focused on questions related to germination temperature and herbicide tolerance will help to improve our understanding of and determine suitable agronomic practices for the crop.

Literature Cited

Amaducci, S., Colauzzi, M., Zatta, A., & Venturi, G. (2008) Flowering dynamics in monoecious and dioecious hemp genotypes. Journal of Industrial Hemp, 13:5-19

Ashton, F.M. & A.S. Crafts. (1973) Mode of action of herbicides. Wiley-Interscience, New York. 504 pp.

Aubin, M. P., Seguin, P., Vanasse, A., Tremblay, G. F., Mustafa, A. F., & Charron, J. B. (2015) Industrial hemp response to nitrogen, phosphorus, and potassium fertilization. Crop, Forage & Turfgrass Management, 1.

Bronaugh, J. H. (2018) Annual report on the status and progress of the industrial hemp research program. Virginia Department of Agriculture and Consumer Services. Available at https://rga.lis.virginia.gov/Published/2018/RD563/PDF.

Carus, M., & Sarmento, L. (2016) The European Hemp Industry: Cultivation, processing and applications for fibres, shivs, seeds and flowers. http://eiha.org/media/2016/05/16-05-17-European-Hemp-Industry-2013.pdf. Accessed 30 April, 2019.

Clarke, R. C., & Merlin, M. D. (2013) *Cannabis*: evolution and ethnobotany. Univ of California Press.

Cromack, H. T. H. (1998) The effect of cultivar and seed density on the production and fibre content of *Cannabis sativa* in southern England. Industrial crops and products, 7: 205-210.

Deitch, R. (2003) Hemp: American history revisited: the plant with a divided history. Algora Publishing.

Douglas-Harper Online Etymology Dictionary. 2019. https://www.etymonline.com/word/canvas. Accessed 4 February, 2019.

Edwards, T.J. (1932) Temperature Relations of Seed Germination. The Quarterly Review of Biology, 7:428-443.

Ehrensing, D. T. (1998) Feasibility of industrial hemp production in the United States Pacific Northwest.

Fernandez-Tendero, E., Day, A., Legros, S., Habrant, A., Hawkins, S., & Chabbert, B. (2017) Changes in hemp secondary fiber production related to technical fiber variability revealed by light microscopy and attenuated total reflectance Fourier transform infrared spectroscopy. PloS one, 12(6), e0179794.

Fike J. (2016) Industrial Hemp: Renewed Opportunities for an Ancient Crop. Critical Reviews in Plant Sciences, 35:5-6, 406-424.

Fortenbery, T. Randall & Bennett, M. (2004) Opportunities for Commercial Hemp Production. Applied Economics Perspectives and Policy. 26:97-117.

Garcia,-Jaldon, C., D. Dupreyre, & M.R. Vignon. (1998) Fibres from semi-retted hemp bundles by steam explosion treatment. Biomass Energy 14:251–260.

Hall J, Bhattarai SP & Midmore DJ. (2014) Effect of industrial hemp (*Cannabis sativa* L) planting density on weed suppression, crop growth, physiological responses, and fibre yield in the subtropics. Renew Bioresour. 2:1. Available at: http://dx.doi.org/10.7243/2052-6237-2-1.

Herndon, G. M. (1963) Hemp in colonial Virginia. Agricultural History, 37, 86-93.

Husbands, J.D. (1909) USDA Bureau of Plant Industry Bulletin 153, 42.

Industrial Hemp Growers Get Access to Edge® Granular Herbicide. (n.d.) Retrieved from http://ca.gowanco.com/news/industrial-hemp-growers-get-access-edge-granular-herbicide Accessed 4 February, 2019.

Johnson, P. (1999) Industrial Hemp: A Critical Review of Claimed Potentials for *Cannabis sativa*. Tappi Journal, 82, 113-123.

Johnson, R. (2018) Hemp as an Agricultural Commodity. Congressional Research Service

Kandel R., Mueller D., Legleiter T., Johnson W., Young B. & Wise K., (2018) Impact of fluopyram fungicide and preemergence herbicides on soybean injury, population, sudden death syndrome, and yield. Crop Protection. 106:103-109.

Karus, M., & Vogt, D. (2004) European hemp industry: Cultivation, processing and product lines. Euphytica, 140(1-2), 7-12.

Khan, M. M. R., Y. Chen, T. Belsham, C. Lagué, H. Landry, Q. Peng,& W. Zhong. (2011) Fineness and tensile properties of hemp (*Cannabis sativa L.*) fibres. Biosystems Engineering. 108:9-17.

Lisson, S. N., Mendham, N. J., & Carberry, P. S. (2000) Development of a hemp (*Cannabis sativa* L.) simulation model 1. General introduction and the effect of temperature on the pre-emergent development of hemp. Australian Journal of Experimental Agriculture. 40:405-411.

Liu, M., Fernando, D., Daniel, G., Madsen, B., Meyer, A. S., Ale, M. T., & Thygesen, A. (2015) Effect of harvest time and field retting duration on the chemical composition, morphology and mechanical properties of hemp fibers. Industrial Crops and Products. 69:29-39.

Mandolino, G., & Carboni, A. (2004) Potential of marker-assisted selection in hemp genetic improvement. Euphytica, 140:107-120.

Maxwell, Brett A., "Effects of Herbicides on Industrial Hemp (*Cannabis Sativa*) Phytotoxicity, Biomass, and Seed Yield" (2016). Masters Theses & Specialist Projects. Paper 1742. https://digitalcommons.wku.edu/theses/1742

Poisa, L., & Adamovics, A. (2010) Hemp (*Cannabis sativa* L.) as an environmentally friendly energyplant. Scientific Journal of Riga Technical University. Environmental and Climate Technologies. 5:80-85.

Pollio A. (2016) The Name of *Cannabis:* A Short Guide for Nonbotanists. *Cannabis* and cannabinoid research, *Cannabis* and Cannabinoid Research. 1:234-238. Available online at: https://www.liebertpub.com/doi/10.1089/can.2016.0027. Accessed 4 February 2019.

Rehman, M. S. U., Rashid, N., Saif, A., Mahmood, T., & Han, J. I. (2013) Potential of bioenergy production from industrial hemp (*Cannabis sativa*): Pakistan perspective. Renewable and Sustainable Energy Reviews. 18:154-164.

Roth, G., Harper, J., Manzo, H., Collins, A., & Kime, L. (2018) Industrial hemp production. *In* Agricultural Alternatives, Penn State University Extension. Available at: https://extension.psu.edu/industrial-hemp-production. Accessed 4 February 2019.

Salentijn, E. M., Zhang, Q., Amaducci, S., Yang, M., & Trindade, L. M. (2015) New developments in fiber hemp (*Cannabis sativa* L.) breeding. Industrial Crops and Products. 68:32-41.

Schultes, R. E. (1970) Random thoughts and queries on the botany of *Cannabis (pp. 11-33). J. & A. Churchill.*

Shaner, D.L., ed. (2014) Herbicide Handbook. 10th ed. Weed Science Society of America, Lawrence, KS.

Small, E., & Marcus, D. (2002) Hemp: a new crop with new uses for North America. Trends in new crops and new uses, 284-326.

Small, E. (2015) Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. Botan. Rev. 81:189-294.

Smartt, J. (1976) Evolution of crop plants. N. W. Simmonds (Ed.). London: Longman.

Tang, K., Struik, P. C., Yin, X., Calzolari, D., Musio, S., Thouminot, C., ... & Amaducci, S. (2017) A comprehensive study of planting density and nitrogen fertilization effect on dual-purpose hemp (*Cannabis sativa* L.) cultivation. Industrial Crops and Products. 107:427-438.

Taylor-Lovell, S., L. M. Wax, & R. Nelson. (2001) Phytoxic response and yield of soybean (*Glycine max*) varieties treated with sulfentrazone or flumioxazin. Weed Technol. 15:95-102.

U.S. Department of Agriculture. (2000) Industrial hemp in the United States: status and market potential. 38pp.

Van der Werf, H.M.G., Van den Berg, W. (1995) Nitrogen fertilization and sex expression affect size variability of fibre hemp (*Cannabis sativa* L.). Oecologia 103:462-470.

Van der Werf, H. M., Wijlhuizen, M., & De Schutter, J. A. A. (1995) Plant density and self-thinning affect yield and quality of fibre hemp (*Cannabis sativa* L.). Field Crops Research, 40:153-164.

Whitford, A. C. (1941) Textile fibers used in eastern aboriginal North America. Anthropological papers of the AMNH; v. 38, pt. 1.

Wilsie, C. P., C. A. Black,& A. R. Aandahl. (1944) Hemp production experiments cultural practices and soil requirements. Bulletin P: Vol. 3: Bulletin P63, Article 1. Iowa State University Digital Repository. Available at: https://lib.dr.iastate.edu/bulletinp/vol3/iss63/1 Accessed 3 April, 2019.

Chapter 2: Industrial hemp germination in response to temperature

Abstract

Industrial hemp (*Cannabis sativa* L.) has reemerged over the past few years as a potential agricultural commodity crop. Research on the crop is limited, and no information exists in the modern peer-reviewed research literature regarding industrial hemp seed germination. Seed germination depends on many factors but among these, knowledge of the response to temperature is important in determining planting date. Eight industrial hemp cultivars from a wide range of latitudes were tested to determine percent germination and absolute germination rate across a range of temperatures. Cultivars were from Northern Europe, Southern Europe, and Canada. Seed were germinated on a thermogradient table; temperatures were maintained from 0 to 45°C in approximately 2°C increments. Base temperature estimates ranged from 0.6 to 4.1°C but these values are inconclusive due to an experimental error. All cultivars germinated at temperatures above 40°C (average maximum temperature = 44.5°C), but average germination percentage above 40 °C was less than 10%. Maximum germination percentages for Canadian and Northern European cultivars (72% and 82%, respectively) occurred at 20°C. The Southern European cultivars had 85 to 92% germination in the 12 to 25°C temperature range. Germination rates were greatest above 20°C, but germination percentages for most varieties began to decline between 25 and 30°C, and fell dramatically at temperatures above 40°C. These data suggest optimum soil temperatures for cultivars from northern latitudes will be in the range of 15 to 20°C given highest germination percentages and moderate germination rates. The optimum may be higher (20 to 25°C) for lines from more southern latitudes given high germination rate and no decline in germination percent in this temperature range.

Introduction

Hemp is a short-day, herbaceous, summer-annual crop adopted from Central North East Asia (El-Sohly 2002, Russo 2001, Russo 2002, Ranalli 2004). Hemp cultivars are broadly adapted to a wide range of environmental conditions (Ehrensing 1998). Asian hemp fiber crops are grown as far south as Yunnan province (24°N). In Europe, hemp cultivars are grown from Finland (>60°N) to Italy (~45°N). Some hemp cultivars can endure both low and high temperatures and seedlings can tolerate some exposure to frost (Fike, personal observation). In the U.S., planting date recommendations for the North-South transition zone generally fall in early May. Optimal timing for planting, seed germination, and stand success may be a function of the interplay between a variety's source of origin and growing conditions such as soil temperature.

Temperature germination research with agronomic crops has been conducted since the late 1800s, but these early germination and growth response measures were lacked accuracy and precision given the limited capacity for setting and tracking temperature (Lisson et. al., 2000). Germination response of hemp was tested in the early 1900s, along with crops such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and white clover (*Trifolium repens* L.). Typically, seed were incubated on a block of ice for 4 months. Germination responses (<10%) were analyzed between crop seeds by Uloth, cited by Edwards 1932. Recent germination research conducted by Lisson et. al. (2000) concluded that optimum and maximum temperatures for radical emergence were 29 and 41°C, respectively, for the hemp cultivar "Kompolti". Base temperatures for radical emergence were variable, ranging from 1 to 6 °C (Lisson et. al., 2000). Van der Werf et al. (1995) reported the base temperature for leaf growth (expansion) was 3 °C and development (leaf appearance) occurred at 1 °C. Based the results of this review, it is hypothesized that the origin of cultivar and temperature of the soil are driving factors in crop germination. The limited literature on hemp seed germination also suggests it has one of the lowest base germination temperature among field crops.

Given the limited number of studies available on hemp germination, the objective of this study was to measure hemp seed germination across a temperature (0 to 45°C) gradient. Because varieties developed across the range of latitudes would experience very different environmental conditions for germination, we hypothesized that germination percentage and rate would vary as a function of variety and latitude of origin.

Materials and Methods

Eight industrial hemp varieties chosen for this study were selected based on their geographic origins (Table 1). The varieties broadly can be described as Canadian, Northern European, and Southern European. Certified hemp seeds were provided to Virginia Tech by the Virginia Department of Agriculture and Consumer Services. All seeds were stored in room temperature in plastic self-sealing bags from the 2018 growing season.

Table 1. Origin, sex type and seed source for eight hemp varieties used to test germination in response to temperature on a thermogradient table.

Region	Country	Cultivar	Type	Source
Canadian	Canada	Canda	Monoecious	Parkland Seed Co.
	Canada	Joey	Monoecious	Parkland Seed Co.
Northern Europe	Poland	Bialobrzeskie	Dioecious	Hemp Exchange
	Ukraine	USO 31	Monoecious	Hemp Exchange
	Ukraine	Zolotonosha	Dioecious	Andrew Kinsel
Southern Europe	France	Felina 32	Monoecious	Schiavi Seeds
	Italy	Compana Elleta	Dioecious	Schiavi Seeds

A linear, insulated, and enclosed thermogradient table (Figure 1) was used to test germination across the range of temperatures (0, 3, 7, 9, 12, 15, 19, 20, 21, 22, 25 27, 31, 33, 38, 40, 41, 43, 45°C) running the germination table set at 0 to 20°C, 20 to 40°C, and 40 to 45°C. The table was constructed with a 6.4-mm-thick x 1-m-wide x 1.2-m-long aluminum plate, under which are welded square metal tubes. A thermal gradient on the metal plate is maintained by pumping cooled or warmed ethylene glycol through the tubes from opposite ends of the table.

Square plastic boxes with moistened germination paper were used to germinate the hemp seed. Eight rows of eight plastic boxes could be placed on the table (Figure 1). All varieties were tested within a row on the table. Petroleum jelly applied on the bottom of each box ensured contact with the gradient table, so that the temperature inside the dish was comparable to the temperature on the table surface. Temperature variance within rows (i.e., along the width of the table) was <1°C, while a 20°C gradient was maintained across the length of the table. In Figure 1, the boxes located at the bottom row of the table were approximately 0°C. The boxes on the top row of the table in the figure are at approximately 20°C. There were a total of 8 rows of boxes that could fit on the table per run. To generate two replicates of each cultivar x temperature combination, runs for each temperature range were conducted twice.

For each test, fifty seeds of each variety were counted and placed into each germination box. Water (20 ml) was added to each box to initiate the germination process. Evaporation of moisture was common in the boxes held at higher temperatures, so more moisture was added in 10-ml increments as needed. Temperatures at the center of each germination box were verified by measuring with a digital infrared thermometer. Boxes were taken from the gradient table when germinated seed were counted and removed in order to facilitate the collection and removal process. Seeds in the boxes were examined at approximately 24 to 72 h intervals and considered germinated at radical emergence. Numbers of seeds germinated and hours after sowing were recorded at each measurement event, and seeds with radicals emerged were removed and discarded. Germination trials were terminated when radical emergence was not observed for a period of 72 h.

A power outage occurred while seeds were approaching germination at the 6 °C temperature mark. Due to this incident, data below 7°C was not included in this analysis.

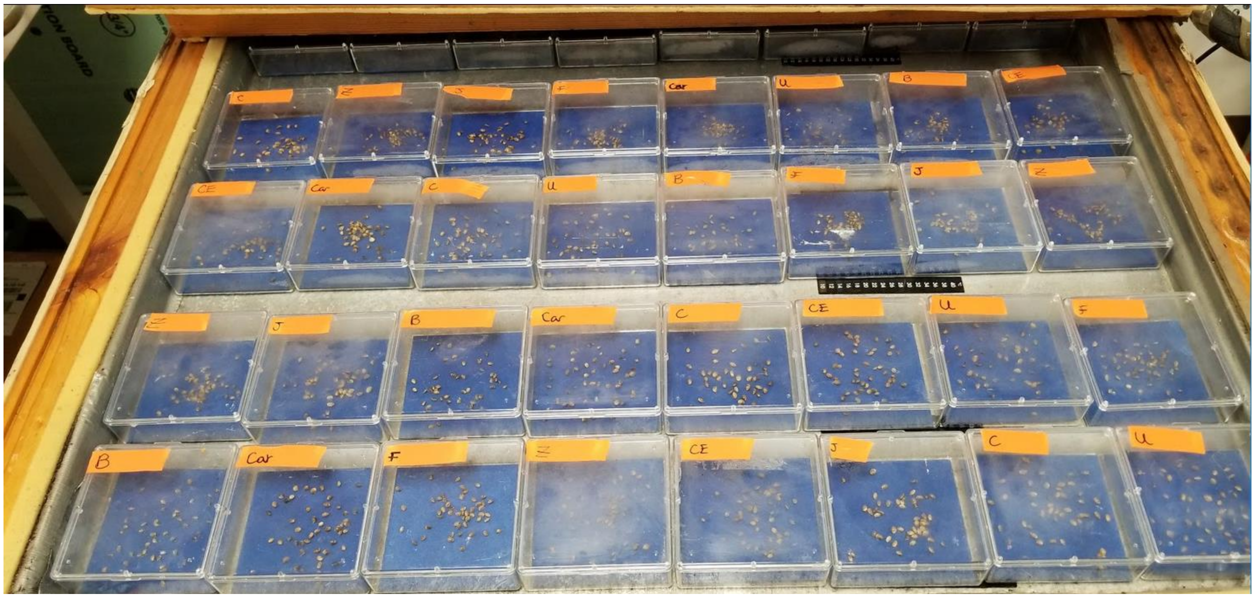


Figure 1. Hemp varieties within plastic boxes on a thermogradient table used to test the effect of temperature on germination % and rate to calculate base and ceiling temperatures.

Germination Data Analysis

Numbers of seed germinated and days after sowing (DAS) to radical emergence were collected and entered in Excel, graphed and visually explored. Mean germination percent responses were graphed against temperature to determine the mean base temperature (T_b) for germination (Scott and Jones, 1985). Given the limited replicates and high variability in germination response, germination data of individual cultivars were combined by region (i.e., Canadian, Northern and Southern European lines of origin). Linear slopes were generated for each region and the intercepts of the regression lines to the abscissa were used to estimate T_b following Gummerson, (1986). However, at temperatures above 20 °C, germination percent, and germination rate (GR) were variable. Optimal germination temperatures (T_o) were based on those values where germination percentage and germination rate were simultaneously highest (Zhou et al., 2015). The highest temperature at which germination occurred was considered the maximum temperature (T_m) of each cultivar (Jett and Welbaum, 1996). Variation in the final germination percent of each region was analyzed using general linear model coefficients with a Poisson regression model (link function: log). Temperature, region, replication, and temperature × region variables was assessed by an analysis of deviance using SAS JMP Pro 14 (Cary, NC).

Results and Discussion

Germination percent. Three general observations about germination percentage could be made from the visual review of the data (Figure 2). Germination percent for seed from all three regions increased from 7 to 12°C and generally remained above 70 % at temperatures up to 20°C. This range of temperatures would be similar to a mid-spring planting window (Pavlisto et al., 2015). Between 12 and 30°C, germination percentage differences became more distinct by region. Southern European varieties had the highest germination, averaging about 90% germination over the 12 to 25°C temperature range. The Canadian and Northern European lines had lower but consistent germination percentages in the 12 to 20°C range, but germination percentage began to decline above about 20°C. In the 22 to 30°C range, germination for Northern European and Canadian lines averaged 67 and 56%, respectively. Hemp from all regions had reduced germination percentages at temperatures above 30°C, although in this range Southern European cultivars have higher germination percentages. At temperatures above 40°C no hemp cultivar had more than 20% germination.

Germination rate. At temperatures less than 10°C, GR was less than 0.12 seeds/day for hemp of all origins, and germination continued for more than 20 days after sowing (DAS) (Figure 3). These measures were gathered only once, however, as a power outage occurred during the second run and the table warmed to room temperature, allowing rapid seed germination. Thus, only data at temperatures above 7°C were used in assessment of GR.

For hemp of all origins, GR doubled between 7°C and 19°C (Figure 3), but rates were low, averaging 0.2 seed/day. A marked shift occurred at temperatures above 20°C, when GR nearly doubled for seed from all regions at temperatures above 20°C. Similar to the germination percent data, rates of radical emergence were more variable above 20°C. The large increase in GR within a narrow range of temperature (from about 19 to 22°C), coincided with (and is confounded by) different runs of the germination table to achieve the different temperature ranges (i.e., 0°C to 20 and 20 to 40°C). The variability of results in this range also likely reflects too large a time interval between observations. At higher temperatures, with faster germination rates, seeds should have been observed every 12 h. Bracketing or overlapping the 20°C and 40°C temperatures from run to run (e.g., runs of 0 to 20°C, 15 to 35°C and 30 to 45°C) may have facilitated better assessment of GR. Despite germination percentages declining rapidly above 40°C (Figure 2), the highest GR (0.9 seeds/day for hemp of Southern European origin) was observed at 45°C, the highest temperature tested.

Base, optimum, and maximum temperatures. Germination percent differed by temperature and region, and significant region × temperature interaction also was observed

(Table 2). Calculated T_b values were 0.6°C (Southern European), 2.6°C (Northern European), and 4.1°C (Canadian). Across all cultivars, T_b calculated averaged about 1°C, which is quite low for any crop species. Edwards (1932) reported signs of hemp seed germination (<10%) 45 DAS when the seed were incubated on a block of ice. Lisson et al. (2000) reported the T_b of hemp cultivar "Kompolti" ranged from 1 to 6°C, which is in agreement with our results. Recent data from the University of Kentucky also suggest T_b is around 4°C (G. Welbaum, personal communication). Germinability at such low temperatures suggests hemp can be established earlier in the season than other common row crops, although success for early planting strategies will need to consider soil moisture and weed management conditions, and the slow rate of germination and limited cover would likely be an issue on erodible soils.

Methodology may also have played a role in the low estimates of T_b because germination boxes were removed from the gradient table to facilitate the observations. Both environment and hormonal conditions affect germination (Welbaum et al., 1990), and in this case brief exposure to higher temperatures when boxes were off the thermogradient table may have provided sufficient warmth to the seed to stimulate germination below the species' true T_b. Both T_b and T_m were determined from graphs of the germination data, and subtle differences or errors in these data can have a significant effect on these estimates (Zhou et al., 2015). No consistent pattern for T_b or T_o was observed among cultivars from the different origins. That is, for the two Canadian and two Southern European cultivars, both low and high T_b were observed.

For all cultivars, highest germination generally occurred between 10 and 20°C. Canadian lines appeared more sensitive to higher temperatures (i.e., had greater reductions in germination) in the 20 to 30°C range. These empirical data suggest a T_o for most cultivars in the range of 20 to 25°C. Counter to our hypothesis, lines developed from Southern Europe did not display greater germination at the highest temperatures. In comparison, Lisson et. al 2000 estimated the optimum and maximum temperatures for radical emergence were 29 and 41 °C for hemp cultivar "Kompolti". All cultivars had some seed germination at temperatures as high as 45°C, although germination percentages fell markedly at about 39 or 40°C. Thus, to determine a true T_m will require testing at temperatures higher than 45°C (Lisson et al., 2000).

Seed quality may have been an additional factor affecting these results. Fungal growth, observed in germination trays across a range of temperatures for USO 31 and Zolotonosha provided clear evidence of poor quality and electrolyte leakage (Simon and Hurron, 1972). This suggests seed membranes are less stable, electrolyte leakage greater, and seed quality will be more important when planting hemp into soils at higher temperatures. Similar findings have been reported for several other species (Bertling et al., 2018). Variations in seed maturity and production environment were not investigated in this study.

Summary and Conclusions

In summary, germination of Canadian, Northern, and Southern European industrial hemp cultivars were tested at different temperatures to generate germination data. Data were affected by experimental error and seed quality issues which precluded accurate determination of T_b. However, other, general conclusions may be inferred. In general, hemp cultivars tested in this study showed a difference in temperature and origin and had a significant interaction term of both variables. Southern European lines appear less sensitive to (have higher germinability at) higher germination temperatures. Germination percentage declines at temperatures above 25°C for Canadian and Northern European lines and at about 30°C for Southern European lines. For all cultivars, germination percentage declined rapidly at temperatures near 40°C, but all cultivars also displayed some limited capacity to germinate at temperatures as high as 45°C. Poisson regression analysis suggests that differences in region of cultivar origin affect hemp seed germination response to temperature. These data require more support but suggest that optimum planting dates relative to soil temperatures is likely to be in an April/May window in Virginia, when soil temperatures are between 15 and 20°C.

Literature Cited

Bertling, A.O. Odindo. O, Manyoni N. 2018. The use of image analysis to assess quality of *Cichorium intybus* seeds differing in coat colour. Horticultural Science, UKZN-Pietermaritzburg, South Africa; Acta Hortic. ISHS 25 – 31

Dewey, L.H. 1901. The hemp industry in the United States. USDA Yearbook, 541-554.

Dewey, L.H. 1913. Hemp. USDA Yearbook, 283-346.

Ehrensing, D. T. 1998. Feasibility of industrial hemp production in the United States Pacific Northwest.

El-Sohly MA. Chemical constituents of *Cannabis*. 2002. In: Grotenhermen F, Russo E, editors. *Cannabis* and Cannabinoids. Pharmacology, Toxicology, and Therapeutic Potential. New York: The Haworth Press: p. 27–36.

Ellis, R.H. and P.D. Butcher. 1988. The effects of priming and 'natural' differences in quality amongst onion seed lots on response of the rate of germination to temperature and the identification of the characteristics under genotypic control. J. Exp. Bot. 39: 935–950.

Gummerson, R.J. 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar-beet. J. Exp. Bot. 37: 729–741.

Jett, L.W. and G.E. Welbaum. 1996. Effects of matric and osmotic priming treatments on broccoli seed germination. J. Am. Soc. Hortic. Sci. 121: 423–429.

Li HL. 1973. The origin and use of *Cannabis* in eastern Asia linguistic-cultural implications. J Econ Bot; 28(3):293–301

Ranalli P, Venturi G. 2004. Hemp as a raw material for industrial applications. Euphytica1 40:1–6.

Russo EB. 2001. Hemp for headache: an in-depth historical and scientific review of *Cannabis* in migraine treatment. J *Cannabis* Ther (2):21–92.

Russo EB. 2002. *Cannabis* treatments in obstetrics and gynaecology: a historical review. J *Cannabis* Ther: 2(3–4):5–34.

Scott, S. J., & Jones, R. A. (1985). Cold tolerance in tomato. I. Seed germination and early seedling growth of *Lycopersicon esculentum*. Physiologia plantarum, 65(4), 487-492.

Simon, E., & Harun, R. R. 1972. Leakage during seed imbibition. J. Exp. Bot. 23:1076-1085.

Welbaum, G.E., T. Tissaoui and K.J. Bradford. 1990. Water relations of seed development and germination in muskmelon (*Cucumis melo* L.). III. Sensitivity of germination to water potential and abscisic acid during development. Plant Physiol. 92:1029–1037.

Zhou, D. Barney, J. Ponder, M. Welbaum, G. (2015). Germination response of Six Sweet Basil (*Ocimum basilicum*) Cultivars to Temperature. Seed Technology. 37:43-51.

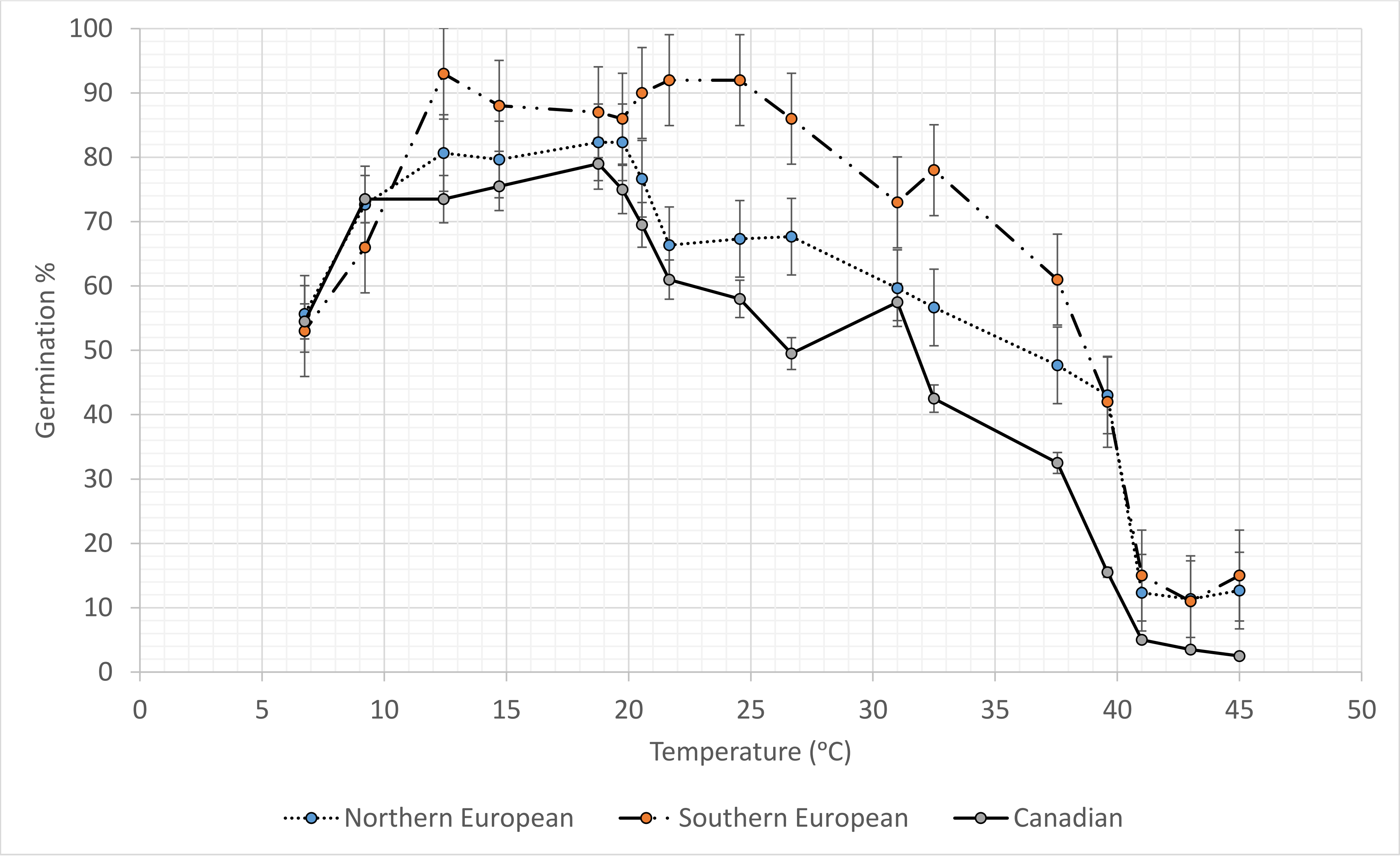


Figure 2: Germination percentages of Canadian, North European, and Southern European cultivars across a range of temperatures with standard error bars.

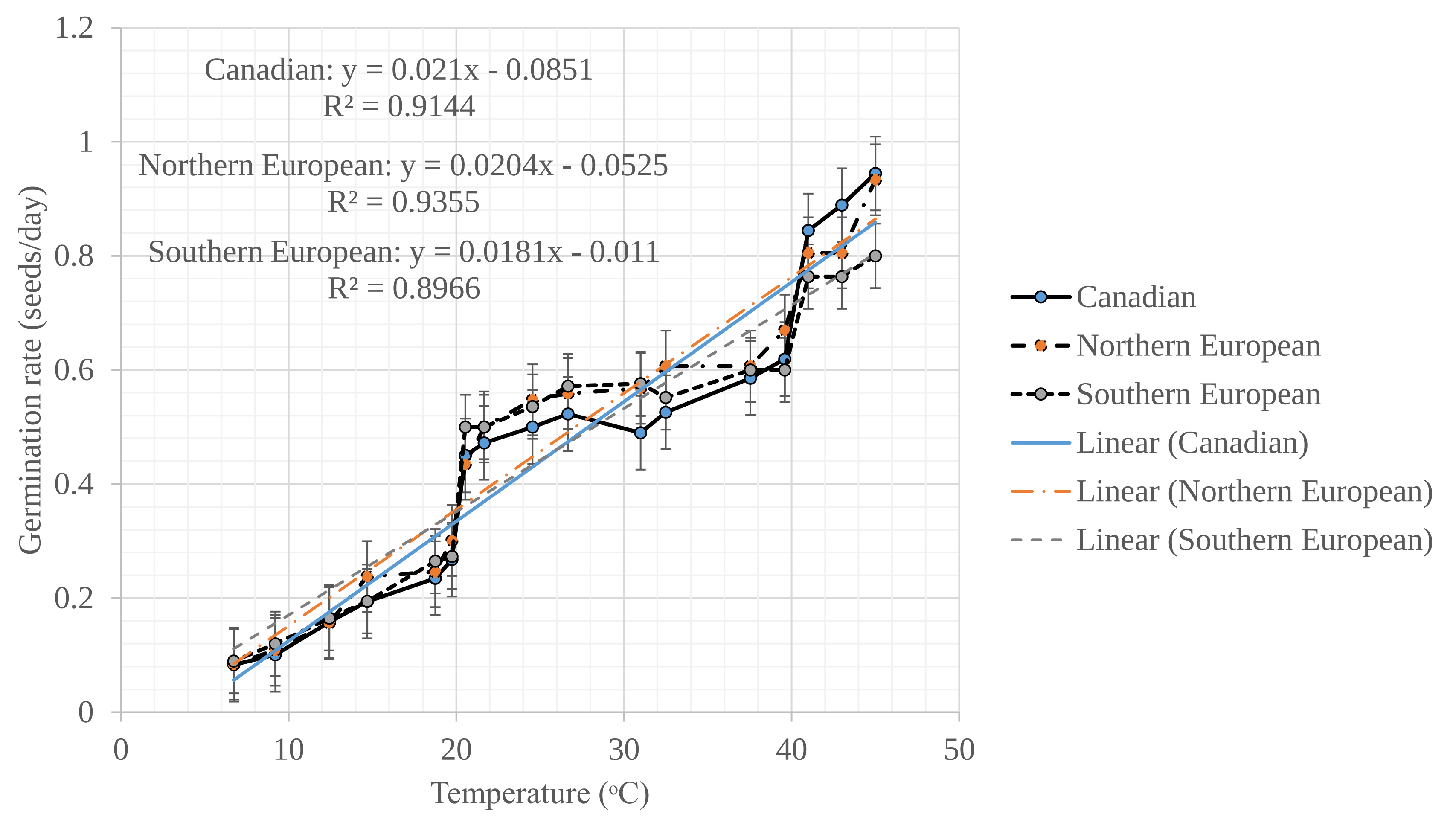


Figure 3: Mean germination rate of six industrial hemp cultivars across various temperatures. Base temperature (T_b) values were estimated from linear regression equations by extrapolating plots of mean GR versus temperature to the x intercept.

Table 2: Effect of temperature and region of the seed source on the germination % of several industrial hemp cultivars.

Variable	DF	L-R† Chi Square	Prob>Chi Sq
Temperature	29	758	<0.001*
Region	2	24.8	<0.001*
Temperature × Region	34	880	<0.001*
Replication	1	0.606	0.436

†Generalized linear regression using the Poisson regression model (link function: log and $n = 238$ observations) of variables was assessed by an analysis of deviance ($ƛ^2$ test).

Chapter 3: Herbicide tolerance screening of industrial hemp (*Cannabis sativa* L.)

Abstract

Industrial hemp (*Cannabis sativa* L.) is a multi-purpose crop that can be used in industries as varied as construction and health. This potential, coupled with substantial reductions in the restrictions on research and production, has spurred renewed interest in hemp in the US over the last 10 years. However, much remains to be investigated to make industrial hemp a sound economic alternative to other crops. At present, little information has been generated regarding suitable pre- and post-emergent herbicides for hemp production, particularly in the eastern US. Thus, the objective of this study was to assess response to various herbicides to identify suitable options for industrial hemp grain or dual-purpose (fiber and grain) production. Preliminary greenhouse experiments with preemergent (PRE) and postemergent (POST) herbicides were conducted to inform herbicide choices for subsequent field trials. The PRE field screening resulted in no differences in grain yields, which ranged from 0.37 to 0.76 Mg ha^{-1}, despite >50% injury 60 days after treatment from chlorimuron, linuron, and pendimethalin. Pendimethalin, and linuron herbicides appear to be suitable PRE options for industrial hemp production as measured by low phytoxicity and acceptable plant growth in greenhouse conditions. In the POST field study, no differences in grain yield were detected relative to the nontreated plots. Yields ranged from 0.28 to 0.74 Mg ha^{-1}. Halosulfuron was the only POST treatment to cause visible injury (70%) relative to the nontreated 30 days after treatment. Among POST treatments, sethoxydim, clopyralid, bromoxynil, and quizalofop applications caused the least injury and resulted in favorable yields (> 0.7 Mg ha^{-1}) that were similar to the nontreated check.

Key words: crop safety, injury, stand reduction, weed management.

Introduction

A reawakening to the versatility and usefulness of hemp for products ranging from engineering fibers and textiles to food and health products has developed in the US over the last 30 years. However, until 2014, use of the plant was limited by Federal restrictions. As of 2016, approximately 30 countries produced hemp as a commodity. This number is expected to rise as the industry continues to grow. In the US, farmers now can grow hemp after passage of the 2018 Farm Bill, but they must make production and management decisions with little basic information, since most hemp agronomic research has been conducted in Europe, Canada, and Asia.

Hemp productivity may be affected by several factors, one of which is weed pressure. Weeds compete for nutrients reducing yield (Peters and Linscott, 1988), crop quality (Oerke, 2006), and harvest ability (Smith et al., 2000). Few studies have focused on weed management strategies for hemp production, and best practices in hemp cropping systems are mostly unknown. Cultural practices that are well established in other crops – including planting date, planting density and spatial arrangement, and crop rotation – are likely to affect stand success and productivity. E.g., wider row spacing used for grain production may support greater crop productivity, but this also may allow for greater weed competition. Little information is available on hemp’s tolerance to herbicides, and currently no herbicides are labeled for use in hemp production in the United States. Currently only a few herbicides are labeled for use with hemp in Canada. Ethalfluralin (Edge® Granular) has added hemp to its label, which is registered for all of Eastern and Western Canada (Gowan Canada, 2018). Quizalofop-p-ethyl (Assure® II Herbicide), is labeled for use in hemp production in Canada (Workflow-Process-Service, n.d.).

Few data have been published regarding hemp's tolerance to different herbicides. Maxwell (2016) applied preemergent (PRE) and postemergent (POST) herbicides to industrial hemp at two sites in Kentucky. Pendimethalin applied PRE caused limited (5%) injury to hemp, while POST herbicides bromoxynil and monosodium methyl arsonate (MSMA) caused only minor (6%) injury. The objective for this research was to test hemp tolerance to PRE and POST herbicides in greenhouse and field studies. Experiments were designed to test the null hypothesis that industrial hemp would not differ in plant injury, growth, and yield responses to various herbicide treatments. Determining hemp's response to PRE and POST herbicides serves a broader objective of developing best management practices to support the development and growth of a potential industrial hemp industry.

Materials and Methods

Experiments were conducted to test industrial hemp tolerance to PRE and POST herbicides in greenhouse and field settings in Blacksburg, VA. Herbicides were chosen for the study based on options commonly used in corn and soybean production and guided in part by previous research conducted in Kentucky. Weed control spectrum is well characterized for these herbicides and therefore was not evaluated in this research. Greenhouse studies were conducted to preliminarily screen herbicides for further testing in the field trials.

Preemergence greenhouse study

Hemp tolerance to 14 different PRE herbicides (Table 3) was tested using a randomized complete block design with eight replications. A nontreated check was also included. A monoecious cultivar ('Felina 32', a dual-purpose French variety) was used. Hemp was grown in pots with Ross silt loam (Fine-loamy, mixed, superactive, mesic Cumulic Hapludoll; NRCS, 2018). Routine soil analyses were conducted prior to study initiation and amended based on recommendations for corn production (Brann et al., 2009). Experimental units (3.78 L plastic pots) were lined with plastic bags to prevent water drainage and possible herbicide leaching, as well as to maintain uniform soil moisture. Plants were watered every three days. Pots were filled by volume with soil and 10 seeds were sown by hand into each pot to a 1 cm depth. Immediately after planting, PRE herbicides were applied in a spray chamber at a rate of 140 L ha^{-1} spray volume with a TeeJet VS8002E nozzle (TeeJet Technologies, Springfield, IL) at 206 kPa. The study was conducted in a greenhouse during periods of increasing day length in the summer of 2017 and repeated in time in 2018.

Following herbicide application, plants were assessed for response to treatments. Response variables included visible injury, total number of live plants (count), plant height, and aboveground dry biomass. Plants were scored for visible injury (plant injury or phytotoxicity) on a 0 (no injury) to 100% (complete plant necrosis) scale (Fehr et al., 1971). Plant heights were measured from the soil surface to the top of each plant in every pot. Average height of living plants within each pot then was calculated. Injury measurements were taken every two wk over an eight wk period. Above ground biomass and height measurements were collected at the final assessment (eight wk after treatment) by cutting plants (dead or alive) 1 cm above the soil surface. Fresh weights of all plants were measured with a field balance and subsamples weighed, dried at 60°C for 48 hr using a forced air oven, and reweighed to determine dry matter concentration.

Postemergence greenhouse study

Individual plants were used to test hemp response to each of 13 POST herbicides (Table 3) in addition to a nontreated check. Plants were grown in 3.8 cm diameter containers (Conetainers™; Stuewe and Sons, Inc., Tangent, OR) lined with plastic bags for reasons previously described. Location, soil, soil amendments, and hemp variety were the same as the PRE greenhouse study, previously described. Seeds (one per container) were planted at 1 cm depth into each of 120 containers.

Herbicide treatments were applied as previously described when plants reached 20 to 28 cm in height. Visible plant injury (%), aboveground biomass (g), plant height (cm) were measured as previously described.

Field studies

To test the effects of PRE and POST herbicides (Table 3) on hemp in a field setting, a third set of experiments was conducted. Due to limited seed availability and therefore space, herbicides selected for the field study were mostly based on top performing treatments in the initial greenhouse work, but limited to one herbicide per site of action or chemical family. The studies were conducted with 'Helena' in 2017; 'Joey' was used for the studies in 2018 because sufficient Helena seed were not available. Both varieties are monoecious, dual-purpose cultivars. Helena was developed in the former Yugoslavia and provided by Schiavi Seeds (Louisville, KY). Joey was developed in Canada and purchased from Parkland Industrial Hemp Growers Co-op, Ltd. (Manitoba, Canada).

Each year, hemp was planted into a tilled seedbed to a depth of 1 cm and with 19-cm row spacing using a drill. In 2017, planting occurred June 5 at a seeding rate of 22.5 kg ha^{-1}. In 2018, planting occurred on June 7, with a 33.7 kg ha^{-1} seeding rate, adjusted to reflect results of separate seeding rate studies. Each year, nitrogen (N) was applied as urea (46-0-0) at a rate of 67 kg ha^{-1}. No additional fertilizers were applied as soil P, K, Ca, and Mg levels were moderate or high in both years.

Each year, experimental plots (1.83 by 3.66 m) were established within areas of the stands that were the most uniformity. Separate experiments were conducted for PRE and POST studies. For the PRE studies, herbicides were applied June 8 each year prior to hemp emergence (three days post planting in 2017 and one day post planting in 2018). POST herbicide applications occurred on July 10, 2017 and July 3, 2018, when hemp was approximately 30 and 25 cm tall, respectively.

Data collected in the PRE study included visible injury at 30 and 60 days after treatment application in both years as previously described. Stand counts were taken 60 days after application. In the POST studies, visible injury data were collected 10, 20, and 30 days after herbicide application.

PRE and POST field experiments were harvested September 15, 2017 and September 7, 2018. Grain yields were obtained using a small-plot combine (Wintersteiger, Ried im Innkreis, Austria). Grain was dried to 8% moisture at 55°C using a forced air dryer to determine final yield values.

Statistical analysis

Analyses of variance (ANOVA) were conducted on all data types in all experiments using JMP software (SAS Institute, Cary NC). Treatment and location (where applicable) were considered fixed effects while replication was considered random. For all studies, means separations for all response variables were conducted using Tukey's HSD for all response variables with $\alpha = 0.1$.

Results and Discussion

Preemergence greenhouse study

No treatment by year interactions were observed for any data type for the POST greenhouse experiments; data were pooled accordingly.

PRE herbicide effects on count data were generally apparent from the first measurement (Table 4). Clomazone and norflurazon caused substantial decreases in the number of plants present, with only a single plant observed 2 wk after treatment and low counts (3 and 2 plants) at the remaining rating dates. At all rating events, sulfentrazone, metribuzin, and flumioxazin decreased the number of plants per pot relative to the nontreated controls. Dimethenamid, fomesafen, and pyroxasulfone all reduced the number of plants relative to the nontreated check at one or more rating dates. Chlorimuron, *S*-metolachlor, diuron, linuron, pendimethalin, and acetochlor had similar stand counts as the nontreated pots at all rating dates.

All herbicides caused at least 15% visible injury at some point during the study (Table 4). Visible injury symptoms increased 4, 6, and 8 wk after application. Clomazone, norflurazon, pyroxasulfone, fomesafen, and metribuzin were more injurious (> 48% visible injury) 4 to 8 wk after treatment than other treatments with predominant symptoms of stand loss and stunting. Chlorimuron, diuron, linuron, and pendimethalin caused <25% injury throughout the study, generally corroborating the stand count data. Effects of these treatments did not differ from flumioxazin or acetochlor 4 to 8 wk after treatment.

Plant heights were not affected by diuron, linuron, dimethenamid, pendimethalin, fomesafen, sulfentrazone, flumioxazin, and acetochlor treatments (Table 5). All PRE treatments reduced biomass relative to controls (mean = 66%), but diuron, linuron, pendimethalin, sulfentrazone, and flumioxazin caused less reduction (33 to 65%) than clomazone, norflurazon, metribuzin, and pyroxasulfone, which resulted in ≥80% decrease relative to controls (Table 5).

Across rating types and dates, diuron, linuron, and pendimethalin were the safest to hemp. Flumioxazin was also among the safest to hemp in terms of visible injury, height, and biomass measures, but the herbicide caused reduced stand counts.

Postemergence greenhouse study

No treatment by year interactions were observed for any data type for the POST greenhouse experiments; data were pooled accordingly. POST treatments did not cause measureable differences in visible injury two wk after application (Table 6), but symptoms were evident by the fourth wk. Bentazon, thifensulfuron, linuron, imazethapyr, fomesafen, halosulfuron and imazaquin caused > 50% injury four wk after application (Table 6). However, response to treatment had changed somewhat by eight wk after application, suggesting that hemp plants recovered from herbicide injury. That is, injury scores were marginally lower for linuron and more so for halosulfuron, whereas injury scores increased for pyrithiobac and acifluorfen. Eight wk after application, hemp appeared least sensitive to quizalofop, bromoxynil, sethoxydim, halosulfuron, and clopyralid treatments, which had < 35% visible injury (Table 6).

Plant heights in response to POST treatments were highly variable (Table 7). Despite some visible evidence of treatment effect, only pyrithiobac, bromoxynil, thifensulfuron, linuron, and chlorimuron treatments significantly reduced plant heights (about 25 to 46% shorter than controls). Similarly, biomass data were variable in response to POST applications (Table 7), although more treatments appeared to negatively affect plant dry matter yield than affected heights. Along with pyrithiobac, bromoxynil, thifensulfuron, linuron, and chlorimuron, hemp treated with bentazon, imazethapyr, imazaquin, pyrithiobac, and bromoxynil treatments weighed on average about 52% less than control plants. Plants treated with quizalofop, clopyralid, fomesafen, acifluorfen, sethoxydim, and halosulfuron were similar to controls both for height and biomass measures.

Across rating types and dates, quizalofop, sethoxydim, and bromoxynil were the safest to hemp. Halosulfuron and clopyralid was also among the safest to hemp for visible injury, height, and biomass data types but did reduce stand count.

Field studies

Total accumulated rainfall and monthly average air temperatures were collected for the period May through September in 2017 and 2018 (Figure 4 and 5, respectively). Total accumulated rainfall in the period under study in 2018 (588 mm) was 41% greater than accumulated precipitation during the similar period in 2017 (Figure 4). In 2017, less rainfall occurred during the vegetative stages and a great increase in rainfall at the end of the season.

Among PRE treatments in 2017, pendimethalin, linuron, and chlorimuron caused greater (>50%) visible injury than fomesafen (~25%) and *S*-metolachlor (5%) (Table 9). Despite differences in visible injury, grain yields were comparable for all PRE treatments; average yield was 0.44 Mg/ha (Table 10). In 2018, hemp treated with pendimethalin had the greatest (50%) injury symptoms 30 d after application. Linuron, *S*-metolachlor, and chlorimuron caused similar (15 to 28%) visible injury at the 30 day mark (Table 9). By 60 days after application, differences in injury were not observed in 2018, and grain yields did not differ among treatments. Transient visible injury indicates the plants recovered from injury, similar to observations from the 2017 experiment. *S*-metolachlor caused the least injury of any PRE for industrial hemp plots. *S*-metolachlor resulted in 90% stand relative to the nontreated check 60 days after application (Table 8). Pendimethalin treatments resulted in the lowest stand counts (Table 8) and high ratings of visible injury (Maxwell, 2014) (Table 9) both years, but had similar grain yield ratings (Table 10). Chlorimuron, fomesafen, and linuron had similar stand counts.

With the exception of halosulfuron, POST treatments had minimal effects on hemp visible injury (Table 9) and grain yield (Table 10) in 2017. Visible injury reached 70% for hemp treated with halosulfuron, and had >25% reductions in grain yields (0.28 Mg ha^{-1}) compared with controls (0.39 Mg ha^{-1}). Similar response to halosulfuron was observed in 2018, with applications causing 59% visible injury 9 d after application. However, grain yields did not differ among POST treatments in 2018. Sethoxydim caused injury symptoms 9 and 21 days after application in 2017 but no injury was analyzed in 2018. In 2017, the sethoxydim treatment was applied following the halosulfuron application, suggesting that application equipment did not get adequately cleaned between these treatments. Sethoxydim, quizalofop, and clopyralid were favorable herbicides that performed well in this study.

Herbicide effects observed in these studies was consistent with previous research. Halosulfuron can cause rapid growth inhibition of broadleaf crops (Vencill, 2002). Postemergent herbicides sethoxydim, bromoxynil, and quizalofop had minimal visible injury during both years of the study. Maxwell (2014) reported that bromoxynil had a displayed acceptable amounts of injury aligning with our research. Burnside et al. (1994) reported that 9 to 11% visual injury from sethoxydim in a broadleaf when it was applied in combination with imazethapyr or acifluorfen and bentazon. These injuries were transient and had no significant effect on yield of that crop (Burnside et al., 1994). Initial crop injury with quizalofop application has been reported to be transient in broadleaf crops and have no adverse effect on morphological characteristics or yield (Soltani et al., 2006).

Considerable variations occurred in hemp visible injury response to preemergent and postemergent herbicides for a future weed control regiment. Results were variable between greenhouse and field studies given that different varieties were used between years. Grain yields from the field study were relatively low making herbicides effects on yield more challenging to detect. Treatments that recorded low values in seed yield with high vegetative injury performed better in seed yield the next year suggesting that hemp tolerance on varietal choice. Our results indicated that all preemergent and postemergent herbicides caused some level of injury that does physically affect the plants during vegetative growth. This strongly indicates industrial hemp is a robust crop that has the ability to recover from injury caused from many herbicides.

Summary and Conclusions

Preemergent and postemergent herbicide applications were tested in indoor and outdoor settings to generate industrial hemp plant injury response data. *S*-metolachlor, pendimethalin, and linuron appear to be suitable preemergent herbicides for industrial hemp production as measured by little visible injury and acceptable plant growth. Chlorimuron and linuron caused significant injury during the vegetative stages but the industrial hemp varieties had favorable yields. Diuron, flumioxazin are favorable pre emergent herbicides that have promise and gave acceptable results. Sethoxydim, bromoxynil, clopyralid, and quizalofop may be suitable postemergent herbicides for industrial hemp production, but some of these treatments did cause some visible injury that was transient in some cases. No differences were observed in both field studies for grain yield. In conclusion, industrial hemp is a robust crop that can tolerate an acceptable amount of injury from various herbicides with different modes of action. Future research must incorporate different cultivars of hemp being tested with herbicides and work to develop management guidelines for weed control in hemp production.

Literature cited

Burnside, O.C., Ahrens, W.H., Holder, B.J., Wiens, M.J., Johnson, M.M., & Ristau, E.A., (1994). Efficacy and economics of various mechanical plus chemical weed control systems in dry beans (*Phaseolus vulgaris*). Weed Technol. 8, 238–244.

Canada, H. (2019). Pest control products (pesticides) acts and regulations. https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/protecting-your-health-environment/pest-control-products-acts-and-regulations-en.html. Accessed 18 February, 2019.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences.

Fehr, W.R., Caviness, C.E., Burmood, D.T., & Pennington, J.S., (1971). Stage of development descriptions for soybeans, Glycine Max (L.) Merrill1. Crop Sci. 11, 929–931.

Frans R E, Talbert R, Marx D, & Crowley H (1986). Experimental design and techniques for measuring and analyzing plant responses to weed control practices. Pages 29–46 in Camper ND, ed. Research Methods in Weed Science. Champaign, IL: Southern Weed Science Society

Gowan, Canada. Industrial Hemp Growers Get Access to Edge® Granular Herbicide. (n.d.). http://ca.gowanco.com/news/industrial-hemp-growers-get-access-edge-granular-herbicide Accessed 18 February, 2019.

Maxwell, B. A. (2016). Effects of Herbicides on Industrial Hemp (*Cannabis sativa*) Phytotoxicity, Biomass, and Seed Yield

NRCS. (2018). Natural Resource Conservation Service. Web Soil Survey. At: http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm Accessed 14 November, 2018.

Oerke, E. C. (2006). Crop losses to pests. The Journal of Agricultural Science, 144, 31-43.

Peters, E.J. & Linscott, D.L. (1988). Weeds and weed control. Alfalfa and alfalfa improvement, American Society Agronomy, Madison, WI., In A.A. Hanson, D.K. Barnes and R.R. Hill (eds), 705-35.

Soltani, N., Robinson, D. E., Shropshire, C., & Sikkema, P. H. (2006). Adzuki bean (*Vigna angularis*) responses to post-emergence herbicides. Crop Protection, 25, 613-617.

Smith, D. T., Baker, R. V., & Steele, G. L. (2000). Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technology, 14, 122-126.

Vencill, W.K. (2002). Herbicide Handbook 8th Edition. Weed Science Society of America, Lawrence, KS. 493 pp.

Workflow-Process-Service. (n.d.). Assure® II Herbicide | DuPont Canada | English. Retrieved February 18, 2019, from http://www.dupont.ca/en/products-and-services/crop-protection/legume-pulse-crop-protection/products/assure-ii.html

Table 3. Pre- and post-emergent herbicides tested for suitability with hemp production.

Active ingredient	Group number	Product rate, kg ae or ai ha^{-1}	Product	Source
Preemergent Herbicides				
Chlorimuron**	2	0.04	Classic	DuPont
Clomazone	13	1.4	Command	FMC
S-metolachlor**	15	1.6	Dual II Magnum	Syngenta
Diuron	7	2.3	Karmex DF	DuPont
Linuron**	7	1.4	Linex 4L	DuPont
Dimethanamid-P	15	0.7	Outlook	BASF
Pendimethalin**	3	1.6	Prowl H2O	BASF
Fomesafen**	14	0.4	Reflex	Syngenta
Norflurazon	12	2.8	Solicam	Syngenta
Sulfentrazone	14	1.8	Spartan 4F	FMC
Metribuzin	5	0.6	TriCor DF	UPL
Flumioxazin	14	0.1	Valor SX	Valent
Acetochlor**	15	3.4	Warrant	Monsanto
Pyroxasulfone	15	0.9	Zidua	BASF
Postemergent Herbicides†				
Quizalofop**	1	1.0	Assure II	DuPont
Bentazon	6	5.6	Basagran	BASF
Bromoxynil**	6	0.3	Buctril	Chipman
Chlorimuron**	2	0.02	Classic	DuPont
Thifensulfuron	2	0.02	Harmony	DuPont
Linuron	7	1.4	Linex 4L	DuPont
Sethoxydim**	1	0.3	Poast	BASF
Imazethapyr	2	0.2	Pursuit	BASF
Fomesafen	14	0.2	Reflex	Syngenta
Halosulfuron**	2	0.05	Sandea	Gowan
Imazaquin	2	0.8	Scepter	AMVAC
Pyrithiobac	2	3.6	Staple	DuPont
Clopyralid**	4	0.1	Stinger	Corteva
Acifluorfen	14	2.2	Ultra Blazer	UPL

†All POST treatments included a surfactant as per product label recommendations.

** indicates herbicides chosen for field study.

Table 4. Plants per pot and visible injury in response to preemergent herbicides in a greenhouse study.

	Weeks after treatment							
Herbicide†	Plants per pot				Visible injury, %			
	2	4	6	8	2	4	6	8
Nontreated	7 a	7 a	7 a	7 a				
Chlorimuron	1 ab	7 ab	7 ab	6 ab	23 a-d	20 cd	17 c-e	18 c-e
Clomazone	6 de	3 cd	3 cd	2 d	18 cd	69 a	78 a	81 a
S-metolachlor	a-c	6 ab	6 ab	5 a-d	23 a-d	41 bc	36 b-d	36 bc
Diuron	6 a-c	6 ab	6 ab	6 ab	15 cd	20 cd	19 de	19 c-e
Linuron	6 ab	6 ab	6 ab	5 a-c	21 b-d	24 c	17 c-e	14 de
Dimethanamid-P	5 bc	4 b-d	5 a-c	5 a-d	32 a-c	39 bc	46 bc	47 b
Pendimethalin	4 a-c	5 a-c	5 a-c	4 a-d	18 cd	23 cd	23 b-e	17 c-e
Fomesafen	1 bc	5 a-d	4 b-d	4 b-d	23 a-d	49 ab	33 b-d	33 b-d
Norflurazon	4 e	2 d	2 d	2 cd	21 b-d	71 a	73 a	90 a
Sulfentrazone	4 cd	4 b-d	4 b-d	4 b-d	24 a-d	38 bc	36 b-d	52 b
Metribuzin	4 c	4 b-d	5 b-d	3 b-d	45 ab	41 bc	40 b-d	34 bc
Flumioxazin	6 cd	4 b-d	4 b-d	3 cd	24 a-d	33 bc	34 b-d	34 b-d
Acetochlor	5 ab	6 ab	6 ab	5 a-d	21 b-d	28 bc	27 b-d	34 b-d
Pyroxasulfone	5 a-c	5 a-c	5 a-d	4 b-d	47 a	50 ab	48 b	46 b
Standard Error	0.55	0.36	0.37	0.37	2.56	4.39	5.04	6.07

† See Table 1 for application rates.

Data were analyzed using Tukey’s HSD. Data were pooled across 2017 and 2018. Differences designated at $\alpha = 0.10$.

Table 5. Hemp height and biomass responses 8 weeks after preemergent herbicide treatment in a greenhouse study.

Treatment[†]	Height, cm/plant	Biomass, g/plant
Nontreated	49 a[‡]	13.5 a
Chlorimuron	24 b-d	4.8 c-f
Clomazone	8 d	0.6 g
S-metolachlor	25 c	5.1 c-f
Diuron	35 a-c	5.7 b-e
Linuron	37 a-c	9.1 b
Dimethanamid-P	39 a-c	5.1 c-f
Pendimethalin	46 ab	7.2 bc
Fomesafen	31 a-c	3.7 d-g
Norflurazon	9 d	1.6 fg
Sulfentrazone	33 a-c	4.7 b-d
Metribuzin	26 cd	2.1 fg
Flumioxazin	41 a-c	6.8 b-d
Acetochlor	32 a-c	4.5 c-f
Pyroxasulfone	21 cd	2.7 e-g
Standard error	3.23	0.82

[†] See Table 1 for application rates.

[‡] Data were analyzed using Tukey's HSD. Data were pooled across 2017 and 2018. Differences designated at $\alpha = 0.10$.

Table 6: Visible injury from postemergent herbicides in a greenhouse study.

	Week after treatment, % visible injury			
Treatment[†]	2	4	6	8
Quizalofop	44 a[‡]	35 ef	37 c-e	27 e
Bentazon	44 a	51 a-e	59 ab	65 ab
Bromoxynil	40 a	33 f	28 e	31 de
Chlorimuron	41 a	37 c-f	43 b-e	40 c-e
Thifensulfuron	38 a	52 a-d	58 ab	58 ac
Linuron	43 a	56 ab	61 a	50 b-d
Sethoxydim	57 a	38 c-f	27 e	27 e
Imazethapyr	49 a	53 a-c	54 a-c	64 ab
Fomesafen	47 a	64 a	60 ab	74 a
Halosulfuron	42 a	51 a-e	32 e	32 de
Imazaquin	56 a	56 ab	65 a	70 ab
Pyrithiobac	38 a	44 b-f	52 a-d	64 ab
Clopyralid	44 a	36 d-f	36 de	33 de
Acifluorfen	56 a	40 c-f	58 ab	61 a-c
Standard Error	1.6	2.6	3.6	4.7

[†] See Table 1 for application rates.

[‡]Data were analyzed using Tukey's HSD. Data were pooled across 2017 and 2018. Differences designated at $\alpha = 0.10$.

Table 7: Hemp height and biomass response to postemergent herbicides in a greenhouse study.

Treatment[†]	Height, cm/plant	Biomass, g/plant
Nontreated	52 a[‡]	3.5 a
Quizalofop	51 ab	3.2 ab
Clopyralid	50 a-c	2.8 a-d
Fomesafen	48 a-d	2.5 a-d
Acifluorfen	48 a-d	2.4 a-d
Bentazon	44 a-e	1.9 c-e
Imazethapyr	44 a-e	1.8 c-e
Sethoxydim	43 a-e	3.0 a-c
Halosulfuron	40 a-e	2.6 a-d
Imazaquin	40 a-f	1.6 de
Pyrithiobac	39 b-f	2.1 b-e
Bromoxynil	39 c-f	1.9 c-e
Thifensulfuron	36 d-f	1.2 e
Linuron	35 ef	1.8 c-e
Chlorimuron	28 f	1.2 e
Standard error	3.23	0.82

[†] See Table 1 for application rates.

[‡] Data was analyzed using Tukey’s HSD. Data were pooled across 2017 and 2018. Differences designated at $\alpha = 0.10$

Table 8. Preemergent herbicide treatment effects on stand count of hemp dual purpose cultivars* measured in field trials in 2017 and 2018.

Treatment by year	Stand Count, plants per 3 m linear row
2017	
Nontreated	18 a[†]
Fomesafen	8 b
Pendimethalin	2 b
Linuron	5 b
S-metolachlor	14 a
Chlorimuron	5 b
Standard error	2.6
2018	
Nontreated	21 a
Fomesafen	22 a
Pendimethalin	10 b
Linuron	25 a
S-metolachlor	20 a
Chlorimuron	20 a
Standard error	2.1

See Table 1 for application rates.

*'Helena' a dual purpose cultivar from Europe was used in 2017. 'Joey', a dual purpose cultivar from Canada was used in 2018.

[†] Means separated according to Tukey's HSD. $\alpha = 0.10$.

Table 9. Pre- and post-emergent herbicide effects on visible injury of dual purpose hemp cultivars* measured in field trials in 2017 and 2018.

Treatment by year	Visible injury, %		
	Days after application		
Preemergent herbicides			
2017	30-DAY	60-DAY	
Fomesafen	55 b[†]	23 b	
Pendimethalin	25 c	73 a	
Linuron	79 a	60 a	
S-metolachlor	0 c	0 b	
Chlorimuron	88 a	70 a	
Standard error	16.4	14.3	
2018			
Fomesafen	10 b	8 a	
Pendimethalin	50 a	20 a	
Linuron	15 ab	10 a	
S-metolachlor	15 ab	8 a	
Chlorimuron	28 ab	5 a	
Standard error	7.2	2.6	
Postemergent herbicides	Visible injury, %		
2017	9-DAY	21-DAY	30-DAY
Clopyralid	5 bc	3 b	0 b
Halosulfuron	61 a	60 a	70 a
Sethoxydim	15 b	13 b	15 b
Bromoxynil	18 b	10 b	4 b
Quizalofop	0 c	5 b	0 b
Standard error	10.9	10.6	13.3
2018			
Clopyralid	5 b	3 b	3 b
Halosulfuron	59 a	63 a	50 a
Sethoxydim	0 b	1 b	7 b
Bromoxynil	0 b	0 b	2 b
Quizalofop	0 b	0 b	1 b
Standard error	11.6	12.3	9.6

*'Helena' a dual purpose cultivar from Europe was used in 2017. 'Joey', a dual purpose cultivar from Canada was used in 2018.

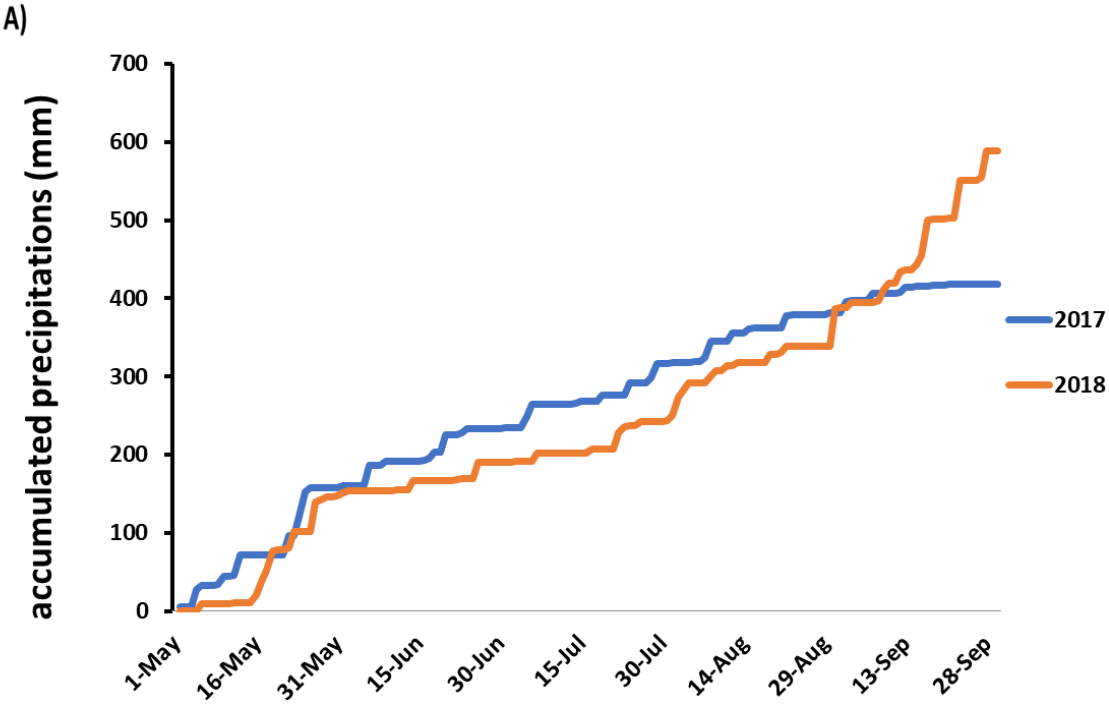
[†] Means separated according to Tukey's HSD. $\alpha = 0.10$.

Table 10. Pre- and post-emergent herbicide effects on grain yield of dual purpose hemp cultivars* measured in field trials in 2017 and 2018.

	Yield, Mg ha^{-1}
Preemergent herbicides	
2017	
Nontreated	0.49 a[†]
Fomesafen	0.47 a
Pendimethalin	0.42 a
Linuron	0.33 a
S-metolachlor	0.51 a
Chlorimuron	0.44 a
Standard error	0.26
2018	
Nontreated	0.67 a
Fomesafen	0.66 a
Pendimethalin	0.75 a
Linuron	0.76 a
S-metolachlor	0.62 a
Chlorimuron	0.74 a
Standard error	0.23
Postemergent herbicides	
2017	
Nontreated	0.39 a
Clopyralid	0.34 a
Halosulfuron	0.28 a
Sethoxydim	0.52 a
Bromoxynil	0.36 a
Quizalofop	0.42 a
Standard error	0.33
2018	
Nontreated	0.74 a
Clopyralid	0.68 a
Halosulfuron	0.48 a
Sethoxydim	0.67 a
Bromoxynil	0.65 a
Quizalofop	0.58 a
Standard error	0.37

*'Helena' a dual purpose cultivar from Europe was used in 2017. 'Joey', a dual purpose cultivar from Canada was used in 2018.

[†] Means separated according to Tukey's HSD at $\alpha = 0.10$



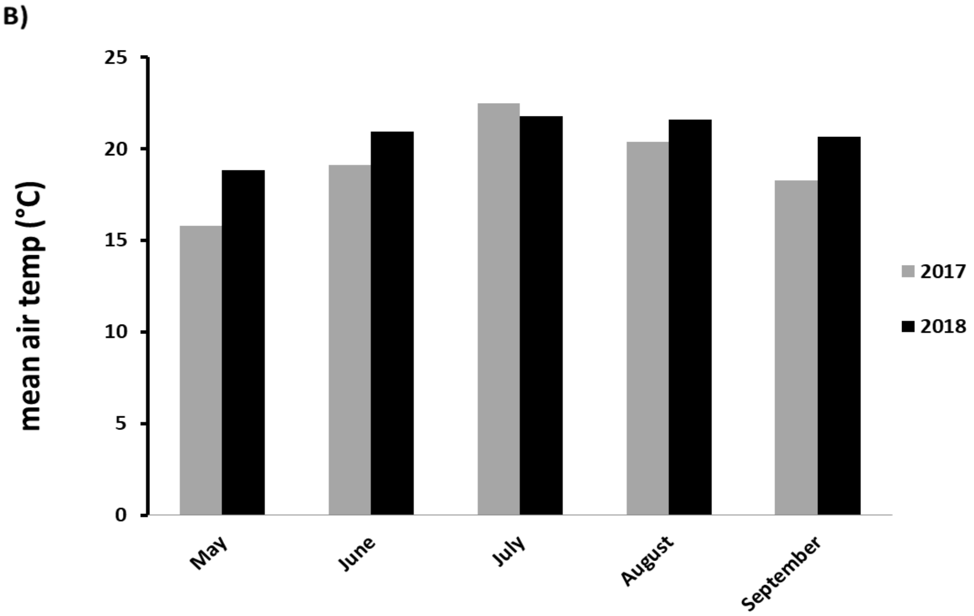


Figure 4) A) Total accumulated rains (mm) (top) and, Figure 5) B) mean air temperatures (°C) (bottom) for the period May through September 2017 and 2018, in Blacksburg, VA. Source: National Oceanic and Atmospheric Administration, National Centers for Environmental Information (https://www.ncdc.noaa.gov).

Chapter 4: Summary and Conclusions

Sowing industrial hemp seeds in the ground in the April – May window is an effective way to influence optimum germination amongst cultivars in the state of Virginia. Our results suggested that the origin of cultivars have a huge influence in their germination response to different environments. Hemp has the ability to germinate in cool and warm soil conditions as differences were observed in germination percent and rate in this study. Germination percentages were lower for some cultivars at high soil temperatures compared to others. Germination rate increased for all cultivars as temperature increased with an increase in rate at the 20 °C mark. Further research should incorporate the use of germination stimulants and analyze germination response based on age differences and contamination.

The application of herbicides with different modes of action on industrial hemp resulted in differences in morphological characteristics and plant injury response. No differences in grain yield were detected suggesting that hemp is a robust crop that has the ability to recover from the injuries caused by herbicides. Plant injury responses were significant for preemergent herbicides 1 and 2 months after application while postemergent effects were witnessed within the first month of application. Overall, our results indicate that S-metolachlor applied as preemergent or sethoxydim, quizalofop, bromoxynil, and clopyralid applied postemergent are suitable candidates for hemp production, but some of these treatments caused transient visible injury. Future research should be conducted to validate results across cultivars, soil types (for preemergent herbicide applications), and environments.