ANNUAL REPORT
ON THE
STATUS AND PROGRESS
OF THE
INDUSTRIAL HEMP RESEARCH PROGRAM

Sandra J. Adams
Commissioner

November 1, 2017
Executive Summary
The 2015 Session of the Virginia General Assembly enacted the Industrial Hemp Law (Law) (Va. Code § 3.2-4112 et seq.), which authorizes the Virginia Department of Agriculture and Consumer Services (VDACS) to establish and oversee an industrial hemp research program that will be directly managed by public institutions of higher education. In 2017, the four public universities with VDACS-approved industrial hemp research programs planted a total of approximately 78 acres in industrial hemp. To facilitate the universities’ research projects, VDACS imported two varieties of industrial hemp planting seed, distributed approximately 314 pounds of planting seed to two of the universities, and issued Industrial Hemp Grower licenses to 34 individuals.
BACKGROUND

The U.S. Congress, through Section 7606 of the Agricultural Act of 2014, allowed state departments of agriculture to grow industrial hemp for research purposes, under certain conditions. The 2015 Session of the Virginia General Assembly enacted the Industrial Hemp Law (Law)1, which authorizes the Virginia Department of Agriculture and Consumer Services (VDACS) to establish and oversee an industrial hemp research program that will be directly managed by public institutions of higher education.

In August 2015, VDACS entered into a memorandum of understanding with James Madison University (JMU), Virginia State University (VSU), and Virginia Polytechnic Institute and State University (Virginia Tech) to conduct industrial hemp research. In 2016, these three research universities planted a total of approximately 37 acres in industrial hemp.

In September 2016, VDACS entered into a memorandum of understanding with a fourth public institution of higher education, the University of Virginia (UVA), to conduct industrial hemp research. In 2017, the four research universities plated a total of approximately 78 acres in industrial hemp.

CONTROLLED SUBSTANCE IMPORTER REGISTRATION

In order for VDACS to import industrial hemp planting seed, the agency must obtain from the U.S. Drug Enforcement Administration (DEA) (i) an importer registration under the federal Controlled Substance Act and (ii) a separate permit for each shipment that the agency imports.

On September 14, 2015, VDACS received its initial Controlled Substance Importer Registration from DEA. Since this date, VDACS has maintained this registration, with DEA most recently renewing VDACS’s Controlled Substance Importer Registration on June 14, 2017, following a site inspection of VDACS’s Seed Laboratory, where VDACS stores the seed it imports, and a review of VDACS’s industrial hemp seed custody records.

HEMP SEED FOR PLANTING

In 2016, VDACS imported 16 varieties of industrial hemp planting seed, which originated in five different countries. At that time, VDACS imported a quantity of the varieties selected by VSU and Virginia Tech to provide planting seed for the 2016 and 2017 growing seasons. In 2017, VDACS imported two additional varieties, totaling approximately 198 pounds, at the request of Virginia Tech and distributed a total of approximately 314 pounds of planting seed to VSU and Virginia Tech. JMU and UVA elected to procure their own industrial hemp planting seed for the 2017 growing season.

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1 Va. Code § 3.2-4112 et seq.
Table 1. Country of origin of the industrial hemp seed varieties imported by VDACS.

<table>
<thead>
<tr>
<th>Country</th>
<th>Seed Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>Delores</td>
</tr>
<tr>
<td>Poland</td>
<td>Tygra</td>
</tr>
<tr>
<td>France</td>
<td>Felina 32</td>
</tr>
<tr>
<td>Italy</td>
<td>Carmagnola</td>
</tr>
<tr>
<td>Ukraine</td>
<td>Glesia</td>
</tr>
<tr>
<td>Serbia</td>
<td>Gliana</td>
</tr>
<tr>
<td>Canada</td>
<td>Joey</td>
</tr>
<tr>
<td>Biedloreski</td>
<td>Wojko</td>
</tr>
<tr>
<td>Fedora 17</td>
<td>Futura 75</td>
</tr>
<tr>
<td>Campana Eletta</td>
<td>Fibranova</td>
</tr>
<tr>
<td>Glesia</td>
<td>USO31</td>
</tr>
<tr>
<td>Serbia</td>
<td>Ferimon (2017)</td>
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<tr>
<td></td>
<td>Zolotonosha 15</td>
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</tbody>
</table>

**INDUSTRIAL HEMP GROWER LICENSE**

The Law requires that, before growing industrial hemp as part of the industrial hemp research program, an individual must (i) have an agreement with a public institution of higher education that documents that the individual will be a participant in that institution’s industrial hemp research program and (ii) obtain a grower license from VDACS.\(^2\) The Law also requires that a state and national fingerprint-based criminal history background check be a component of the application process for this license.\(^3\) As of September 30, 2017, 35 individuals had an active Industrial Hemp Grower License.

Table 2. Number of licensed industrial hemp growers.

<table>
<thead>
<tr>
<th>University conducting hemp research</th>
<th>Number of licensed industrial hemp growers participating in the university’s hemp research during the 2017 growing season</th>
</tr>
</thead>
<tbody>
<tr>
<td>James Madison University</td>
<td>12</td>
</tr>
<tr>
<td>University of Virginia</td>
<td>8</td>
</tr>
<tr>
<td>Virginia State University</td>
<td>3</td>
</tr>
<tr>
<td>Virginia Tech</td>
<td>12</td>
</tr>
</tbody>
</table>

**UNIVERSITIES CONDUCTING RESEARCH**

In August 2015, VDACS entered into a memorandum of understanding (MOU) with JMU, VSU, and Virginia Tech to conduct industrial hemp research. In June 2016, VDACS renewed each university’s MOU for a second year. In September 2016, VDACS entered into an MOU with UVA to conduct industrial hemp research.

While there is no specific industrial hemp funding available through VDACS for those public institutions of higher education that enter into these MOUs, VDACS received an annual appropriation of $75,000 for research, development, and applied commercialization of specialty crops that it elected to allocate to industrial hemp research in fiscal year 2017. Each of the four research universities received $18,750 from VDACS. This funding was eliminated from the fiscal year 2018 budget.

In 2017, the industrial hemp research universities planted a total of approximately 78 acres in industrial hemp by July. JMU, UVA, and Virginia Tech worked with select farmers to grow

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\(^2\) Va. Code § 3.2-4115(B)
\(^3\) Va. Code § 3.2-4115(C)
industrial hemp on privately-owned land. UVA and Virginia Tech also grew industrial hemp on university-owned or university-managed property, as did VSU. In 2016, industrial hemp was grown on production fields in Albemarle, Chesterfield, Montgomery, Nottoway, Orange, and Rockingham counties. In 2017, industrial hemp production fields were added in Caroline, Fluvanna, Hanover, Pittsylvania, and Wise counties.

Table 3. Location of production fields each university planted in industrial hemp in 2017.

<table>
<thead>
<tr>
<th>James Madison University</th>
<th>University of Virginia</th>
<th>Virginia State University</th>
<th>Virginia Tech</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albemarle</td>
<td>Albemarle</td>
<td>Chesterfield</td>
<td>Caroline</td>
</tr>
<tr>
<td>Fluvanna</td>
<td>Hanover</td>
<td>Montgomery</td>
<td></td>
</tr>
<tr>
<td>Rockingham</td>
<td>Wise</td>
<td>Nottoway</td>
<td>Orange</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pittsylvania</td>
</tr>
</tbody>
</table>

As the universities’ MOUs establish the option to renew the research agreement annually for up to five years, at the time of this report, none of the universities have definitive findings to report. Each university submitted a progress report on its research to VDACS. A summary of each report is provided below, and the reports are included in the appendices of this report.

James Madison University

JMU is researching how effectively industrial hemp grows in Virginia’s Ridge and Valley region and is testing whether industrial hemp seed can be planted and harvested with conventional agricultural equipment. JMU is also investigating the use of industrial hemp seed oil in the production of biofuels.

In 2017, JMU partnered with private farmers to grow industrial hemp in Albemarle, Fluvanna, and Rockingham counties. JMU studied five hemp varieties, one of which is a seed variety, three of which are fiber varieties, and one of which is a dual purpose variety. JMU’s preparation and planned maintenance of each production field varied, and JMU determined that planting dates may be a factor in the weed management for hemp crops and that this warrants further research.

JMU determined that its 2016 research demonstrated that (i) hemp productivity can be improved with appropriate levels of nitrogen fertilizer, (ii) effective weed control strategies are essential for organic hemp producers, and (iii) soils that are continuously wet and heavy with clay will be less productive for industrial hemp compared with sandy loam soils that are well-drained.

JMU expected to complete harvest of its 2017 production fields by mid-September, after which it will analyze this year’s data. JMU has observed very few issues with insects, diseases, birds, or other pests. JMU also noted that, during pollination season, many native pollinators, especially bumblebees, were present in the field.

See Appendix A for the complete report submitted by Dr. Michael Renfroe, the principal investigator for JMU’s industrial hemp research.
**University of Virginia**

UVA is conducting a combination of field-based agronomic experiments, greenhouse-based molecular breeding and plant propagation experiments, and laboratory-based plant cell and tissue culture work with the long-term goal of developing regionally-adapted low or no tetrahydrocannabinol (THC) containing industrial hemp cultivars. UVA-Wise is comparing the agronomic performance of industrial hemp varieties to determine which are best-suited for growth in the Southwest region of the Commonwealth, including a study of the varieties’ capacity to sequester heavy metals, leading to phytoremediation.

In 2017, UVA partnered with a private farmer to grow hemp in Hanover, Henrico, and Albemarle counties. UVA planned to plant six varieties of industrial hemp, three of which are fiber varieties and three of which are oil/seed varieties. Due to the weed pressure on the hemp planted at the Hanover production field under a no-tillage approach on June 10, UVA elected to replant hemp at the Hanover production field on July 10 and to forego planting industrial hemp at its planned Henrico production field. Weed pressure again resulted in UVA’s decision to terminate the trial at its Hanover production field. UVA planted industrial hemp at its Albemarle production field on July 13 and observed reasonable growth for five of the six varieties planted. At the time of its progress report, UVA was still evaluating plant density, plant height, stalk diameter, fiber yield, and grain yield data.

UVA also began greenhouse and laboratory studies into best practices for the growth of high cannabidiol (CBD) varieties under greenhouse conditions and the development of expertise in traditional and molecular breeding to create new industrial hemp varieties. At the time of its report, UVA was growing seven CBD varieties known to produce high concentrations of CBD to maturity for the study of the plants’ leaf tissues, floral parts, and seeds.

On June 29, UVA-Wise planted the six varieties of industrial hemp planted at UVA’s production fields in Hanover and Albemarle as well as a seventh variety specifically adapted for phytoremediation. Limited rainfall and aggressive weed growth limited the growth of industrial hemp planted at the production field in Wise County. At the time of its report, UVA-Wise planned to harvest by hand the plants that did grow in order to collect data on these individual plants that may represent a hardy gene pool. UVA-Wise also plans to conduct a detailed analysis of the heavy metal contents in the roots, stems, leaves, flowers, and seed of the industrial hemp plants that did grow.

See Appendix B for the complete report submitted by Dr. Michael Timko, the principal investigator for UVA’s hemp research, with collaboration by Dr. Ryan Huish at UVA-Wise.

**Virginia State University**

VSU is researching and analyzing required soils, growing conditions, and harvest methods related to various varieties of industrial hemp; conducting industrial hemp seed research; and studying the use of industrial hemp in new energy technologies.
In 2017, VSU conducted three studies at its Research and Demonstration Farm (Randolph Farm) in Chesterfield County using a total of 12 varieties of industrial hemp. Under its variety trial, VSU grew six varieties of industrial hemp for grain, five varieties for fiber, and one variety for both grain and fiber. VSU’s second study was a planting date study. For this study, VSU used five fiber and four grain varieties and planted the varieties at two week intervals from mid-April through mid-June. VSU’s nitrogen study evaluated a grain and a fiber variety. VSU noted observable differences in seedling emergence and plant density among the varieties and planting dates studied and plans to make recommendations on adapted varieties, optimum fertilizer rates, and planting dates based on the data it collects over a three to four-year period. VSU also noted the need for comprehensive research by an entomologist and crop pathologist to study industrial hemp pest infestation and diseases.

See Appendix C for the complete report submitted by Dr. Maru Kering, the principal investigator for VSU’s hemp research.

Virginia Polytechnic Institute and State University

Virginia Tech is conducting agronomic trials to identify industrial hemp varieties that are well-suited to the soils and climate of Virginia and to compare yield responses to various agronomic treatments. Virginia Tech is conducting its research at the following Virginia Tech-owned or managed properties: Kentland Farm in Montgomery County, the Northern Piedmont Center in Orange County, and the Southern Piedmont Agricultural Research and Extension Center in Nottoway County. In 2017, Virginia Tech also collaborated with private farmers in Caroline and Pittsylvania counties and a private research laboratory in Montgomery County.

As in 2016, Virginia Tech conducted industrial hemp variety trials at its production fields in Montgomery and Orange counties. Virginia Tech’s 2017 variety trials studied a total of 20 fiber and grain varieties of industrial hemp. Virginia Tech also conducted a planting date study at its production field in Montgomery County using four planting dates. In this study, Virginia Tech will compare crop productivity and yield.

In 2017, Virginia Tech added a crop injury study to its research. Virginia Tech applied pre-emergent herbicides to a production field under fiber management 24 hours after planting and post-emergent herbicides when the plants reached approximately 11 inches in height. Virginia Tech plans to collect fiber biomass and grain yield data as part of this study.

Virginia Tech also added a cannabidiolic acid extraction study to its research in 2017 via a collaboration with Synthonics, a specialty pharmaceutical company whose work is focused on metal coordinated pharmaceuticals. Virginia Tech grew one variety of industrial hemp in a greenhouse setting, and Synthonics extracted cannabinoids from the plants grown in efforts to develop a cannabinoid purification process.

After observing the significant presence of insects on much of the industrial hemp grown by Virginia Tech in 2016, the university added two entomological studies to its 2017 research. One study is assessing the amount of damage inflicted by the Japanese beetle, tarnished plant bug,
brown marmorated stinkbug, and corn earworm. The second study is assessing pest damage and the use of a broad-spectrum insecticide.

At its production field in Nottoway County, Virginia Tech studied a single variety grown for grain, fiber, and dual purposes to explore fertility management and the effects of nitrogen on yield.

See Appendix D for the complete report submitted by Dr. John Fike, the principal investigator for Virginia Tech’s hemp research, with collaboration from Dr. Carol Wilkinson.

**TETRAHYDROCANNABINOL (THC) TESTING**

The Commissioner of Agriculture and Consumer Services is responsible for providing random testing of industrial hemp for compliance with THC levels. Section 7606 of the Agricultural Act of 2014 establishes that industrial hemp grown for research purposes pursuant to this section shall have a THC concentration not more than 0.3 percent on a dry weight basis. At VDACS’s request, the Department of General Services’ Division of Consolidated Laboratory Services (DCLS) identified and validated a test method it is using to determine the THC concentration of industrial hemp samples collected by VDACS inspectors.

In 2016, VDACS collected a sample of each industrial hemp variety grown at each of the universities’ production fields and submitted these samples to DCLS for testing. At the time that last year’s progress report was published, test results were pending for all of these samples. This report will summarize the THC testing conducted on the industrial hemp grown in 2016. DCLS tested 23 samples, and the THC concentration of the majority of the samples collected was less than 0.10 percent. One sample had a THC concentration over 0.3 percent. The variety whose sample had a THC concentration over 0.3 percent was grown on multiple production fields, and the samples of this variety collected from all but one production field had a THC concentration of less than 0.10 percent. In an effort to determine the potential reasons for this anomaly, VDACS consulted with a hemp regulator in another state as well as the seed supplier. VDACS also attempted to resample this variety, but, at the time of VDACS’s site visit, the university had harvested the plants and no plant material appropriate for sampling in accordance with VDACS’s established protocol remained. VDACS was unable to reach a conclusive explanation for this test result, and, ultimately DEA collected the remnants of the subject sample to conduct its own testing. VDACS has not received the results of DEA’s testing as the time of this report.

**2017 REQUEST FOR PROPOSALS**

On May 24, 2017, VDACS issued a third Request for Proposals (RFP), RFP number 301-17-120, seeking public institutions of higher education interested in conducting industrial hemp research. The RFP asked that the proposals indicate the institution’s intended area of research, experiment procedures, and plot location and size. VDACS did not receive any proposals in response to this RFP.

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4 Va. Code § 3.2-4115(K)
2018 Growing Season

The researchers’ preliminary plans for the 2018 growing season indicate that they all plan to work with private farmers to grow industrial hemp for their research. In advance of the 2018 growing season, VDACS will work with the research universities to import hemp seed, as requested by the universities. The agency also will issue new and renewal industrial hemp grower’s licenses, as appropriate.
INVESTIGATION OF INDUSTRIAL HEMP
FOR OIL AND BIOFUEL PRODUCTION IN VIRGINIA

Annual Report to
Virginia Department of Agriculture and Consumer Services

by
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Department of Biology
James Madison University
Harrisonburg, VA 22807

August 30, 2017
Research on the growth of industrial hemp was conducted by James Madison University in cooperation with contract farmers as permitted by the federal Agricultural Act of 2014 and the Virginia Industrial Hemp Farming Act of 2015. Experiments were designed to address the following objectives:

**Field based research** (Dr. Renfroe and contract growers)

1. To determine how effectively hemp will grow in Virginia in the Ridge and Valley Province, examining growing conditions and analyzing soil properties
2. To analyze harvest methods and determine if conventional agricultural equipment (seed drillers and combines) can plant and harvest industrial hemp without major problems
3. To investigate the use of hemp seed oil in the production of fuels (biodiesel) and the use of press cake for livestock feed

**University based research** (Dr. Morton)

1. To explore the potential for the chemical conversion of hemp oil to produce a qualified quality biodiesel
2. To determine the potential for lignocellulosic derived biofuels
3. To develop a strong and extensive life-cycle systems overview

**Site Selection**
To address these objectives four sites were selected for growing hemp. Two sites were upland sites with clay soil located in Albemarle County, VA. One site was in previous cultivation, the second was previously in pasture. Another site was in Fluvanna County, in a lowland site that was a heavy clay soil along a river bottom. The last site was a river bottom with sandy loam located in Rockingham County, Va. Sites were selected based on the participation of qualified farmers with appropriate agricultural equipment, experience, and a location that was not easily accessible by public, and where the crop could be visually screened. All participants in this research were properly licensed by the Virginia Department of Agriculture and Consumer Services.

**Seed**
Industrial hemp seed were imported from an international distributor in accordance with DEA regulations, US Customs, the USDA, and VDACS. Five cultivars were included in our tests, including one seed cultivar, one dual purpose cultivar and three fiber cultivars.

**Albermarle Site 1**
Two fields totaling approximately twelve acres were planted in industrial hemp in Albemarle Co. in early June (Figure 1). This farm is operated as an organic farming operation. The site had been prepared by disking several times, followed by an application of poultry litter at a rate of 2 tons/Ac in May. Seeds were planted using a grain drill. One cultivar was planted at a rate of 42 lb/Ac. The other field was planted at at rate of 29 lb/Ac.
Rainfall was scarce throughout the growing season. There was significant weed pressure in both fields. Hemp grew, but was reduced in height due to the droughty conditions, generally not exceeding 4-5 feet in height. Pigweed, lambsquarters and Jimson weed were abundant in the field. The cultivars at this location were fiber cultivars. Fresh weight and height data were collected from one square meter sample plots across the field.

**Albermarle Site 2**
Approximately 6.4 acres were planted at this site (Figure 2). This site was converted pasture. The field was prepared by discing and rototilling in April followed by application of two tons/Ac of poultry litter in early May. Hemp was planted at a rate of 48 lb/Ac with a fiber cultivar. There was limited rainfall at this site over the course of the growing season. Weed pressure was high in the field. Hemp plants achieved good height ranging from 3-7 feet in height. Prior to planting, liquid manure was applied as fertilizer. Fresh weight and height data were collected from one square meter sample plots across the field.

**Fluvanna Site**
Approximately 5.5 acres were planted at this site (Figure 3). The field had two discing cultivations in April, followed by the application of two tons/Ac of poultry litter in early May. This site had a sloping grade with a high clay percentage in the soil. The lower side of the field stayed moist. Overall, rainfall was scarce over the growing season. Hemp was planted at a rate of 29 lb/Ac with a fiber cultivar. There was limited rainfall at this site over the course of the growing season. Water availability across the field had a large effect on yield, with the better drained portion producing more hemp per square meter than the wetter portions of the field. Hemp plants grew to about 6-8 feet tall in the better portions of the field with some plants reaching over 9 feet in height, but were short in the wetter parts of the field. Fresh weight and height data were collected from one square meter sample plots across the field.

**Rockingham Site**
This field was sprayed with herbicide in early April to clear vegetation. Liquid manure was injected at a rate of 4,500 gallons per acre below the soil surface prior to planting. The field was cultivated by discing and rolling to smooth the field. Two cultivars were planted at this site. One cultivar was an oilseed cultivar planted at 38 lb/Ac and the other was a dual purpose oilseed and fiber cultivar (Figure 4) planted at 41 lb/Ac. These fields have yet to be sampled because they are growing later into the season to produce seeds. Fresh weight and height data will be collected from one square meter sample plots across the field. Additionally, seeds will be collected from plants growing on the sample plots.

**General Observations**
Planting dates may be important to the production of industrial hemp. Hemp can be planted early to get quick canopy closure to help control weeds. However, under droughty conditions, weeds may still become an issue. Later planting dates may allow farmers to control weeds post emergence before planting hemp. A quick start to a late-planted hemp crop may prove beneficial for weed management. These aspects need further research.

Different hemp cultivars vary greatly in degree of monoecy and dioecy. Dioecous pollen producing plants will bear no seeds and die off early, thus contributing little to no stem product
either. Farmers should pay attention to the extent to which cultivars are monoecious as this will have implications for planting rates.

Weeds present among the various fields included the following: redroot pigweed (*Amaranthus retroflexus*), spiny pigweed (*Amaranthus spinosus*) and lambsquarters (*Chenopodium album*) were especially abundant along with lesser amounts of Jimsonweed (*Datura stramonium*), Johnson grass (*Sorghum halapense*), velvetleaf (*Abutilon theophrasti*), morning glory (*Ipomoea purpurea*), ragweed (*Ambrosia artemisiifolia*), annual fleabane (*Erigeron annuus*), pokeweed (*Phytolacca americana*), and other short herbaceous weeds. The only insect pests observed on the hemp in any number were Japanese beetles and June bugs. They fed on anthers primarily and to a very small extent on leaves.

**Products**

We expect to harvest seeds and stems from our fields. Seeds will be processed to produce oil and press cake. Hemp oil will be converted into biodiesel. Press cake will be fed to non-commercial livestock. Hemp stems will be baled and stored for further experimentation.

**Progress**

Our first year of research demonstrated that hemp productivity can be improved with appropriate levels of nitrogen fertilizer. For organic hemp producers, effective weed control strategies are essential. Soils that are continuously wet and heavy with clay will be less productive for industrial hemp compared with sandy loam soils that are well drained.

Our second year research is still underway. We expect to complete harvests by mid-September, after which this year’s data will be analyzed. We observed very few issues with insects, diseases, birds, or other pests. During pollination season, many native pollinators, especially bumblebees were present in the field. Industrial hemp appears to be a good pollen source for native pollinators. Planning is underway for the 2018 planting season.
Figure 1. Hemp in first Albemarle field site. Field collections being made from sample plots. Grasses and weeds present with hemp.

Figure 2. Hemp in second Albemarle field site. Height variability was present across field. Highly monoecious cultivar for dual use.

Figure 3. Hemp in Fluvanna field site. Highly monoecious cultivar for dual use.
Title of Project: The University of Virginia Industrial Hemp Pilot Research Project

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Summary: On May 25, 2016, the Virginia Department of Agriculture and Consumer Services (VDACS) issued a second Request for Proposals (RFP), RFP number 301-16-124, seeking public institutions of higher education interested in conducting industrial hemp research. The University of Virginia (UVA) submitted a research proposal to VDACS in response to this call, and VDACS accepted UVA’s proposal. On September 26, 2016, UVA and VDACS entered into a Memorandum of Understanding (MOU) allowing the University to conduct industrial hemp research. This MOU was renewed on July 26, 2017.

Research at the University of Virginia is licensed by VDACS, the US Drug Enforcement Agency (DEA), and Virginia Board of Pharmacy (VBP) under the following permits:

Industrial Hemp Growers License Number: 2017-03-UVA-031
DEA Registration Number: RT0519201
VA Board of Pharmacy Controlled Substance Registration: 0220001838

The industrial hemp research being conducted by UVA is comprised of a combination of field-based agronomic experiments, greenhouse-based molecular breeding and plant propagation experiments, and laboratory-based plant cell and tissue culture work with the long-term goal of developing regionally-adapted certified low or no THC containing Virginia hemp cultivars with multipurpose uses across the agricultural, manufacturing, energy, and medical fields. A component of this work is the specific modification of the biological properties of the hemp plant to provide fiber, seed, and other derivative materials to support the development and economic growth of a new vertically integrated hemp industry.

The work at the University of Virginia is being sponsored and financially backed by 22nd Century Group, Inc., a plant biotechnology company with a long-term focus on research, development, licensing, manufacturing, and worldwide sales and distribution of agricultural products.
EXPERIMENTAL PLAN AND RESEARCH

Objective 1. Field evaluation of industrial hemp varieties

The overarching goal of these research studies was to identify industrial hemp varieties that could serve as the basis of a genetic improvement program aimed at developing high-performing, regionally-adapted varieties for the Commonwealth of Virginia. Our long-term aim is the release of certified low- or no THC cultivars that can be readily available to farmers throughout the Commonwealth. To the extent possible we also wanted to test and develop standard “farmers’ practices” so that our appraisal would reflect the real potential for growth of our selected cultivars in the Commonwealth. Thus, our experimental approach was minimal and direct.

This report is being submitted prior to the completion of the first growing season so all of the information should be considered as preliminary. Final data will be made available to interested parties upon request.

Field site selection and preparation.

To ensure that a diversity of growing environments and soil conditions were evaluated, three field locations were initially targeted for planting trials of our selected industrial hemp varieties in the 2017 growing season. These sites were: Cabin Hill Farm (in Hanover County), Tree Hill Farm (in Henrico County) and Morven Farms (in Albemarle County). A fourth site was later added on the campus of UVA-Wise (in Wise County).

The selected field plots at Cabin Hill Farm (Hanover), Tree Hill Farm (Henrico) and Morven Farms (Albemarle) were all fields that were currently in crop production, and slated to be planted in either corn or soybean. The planting history of each site is known. Following discussion and on the recommendation of our grower, Mr. Kevin Engel of Cabin Hill Farms, the decision was made to use a no-tillage farming approach to reduce the potential for soil erosion and to keep the production costs (i.e., fuel, labor, and machinery) as low as possible. No-tillage production requires the use of herbicides to control weeds and a heavy seeder capable of penetrating residue and placing seed at a constant depth. Weed control is very important for no-tillage production systems especially if there are persistent perennial weeds.

At each of the three sites, prior to planting the fields, the fields were bush-hogged and then sprayed with the herbicide Roundup (glyphosate) at 3 pints/acre application rate in order to kill off existing vegetation. At the present time no herbicides and pesticides are listed for use in industrial hemp production in the Commonwealth of Virginia and we were cautioned by our seed supplier that in their experience glyphosate residue could have a negative effect on hemp growth. Approximately 2 months elapsed between the burndown of weeds and planting, so we feel that there was little if any residue left from the herbicide treatment and therefore little to no effect on plant growth. Just prior to planting the fields were disked and then finished (smoothed down) using a TurboTill in order to prepare the seed bed.

Of the four selected sites, only the fields at Cabin Hill Farm had access to irrigation and so as a production variable it was not possible to compare irrigated and unirrigated plots at all the sites.
The plots at Cabin Hill Farms received irrigation at the rate of 7/10 inch approximately twice a week, whereas all other sites relied on rainwater for plant growth.

Nitrogen, phosphorus (DAP, diammonium phosphate) and potassium (potash) were applied at 100 lbs per acre before planting at Cabin Hill Farm, Tree Hill Farm and Morven Farms. The fields at UVA-Wise were treated in a distinct manner (see description below).

Seed Materials

A basic requirement for any breeding program is the ability to conserve seed from season to season and to follow the adaptability of genotypes under field selection. Therefore, in partnership with 22nd Century Group, Inc we identified germplasm that had a prior growing history in an agro-ecological and climatic zone 7 similar to that found in Virginia and provided us as researchers the freedom to operate and save seed each season. The material was a combination of varieties with origins domestically and abroad and these varieties are a combination of fiber types, oil /seed types and dual purpose lines. Six varieties were slated for planting in the trials at Cabin Hill Farm, Tree Hill Farm and Morven Farms, and a seventh variety (specifically adapted for phytoremediation) was included at Wise (see description below). The seeds were transferred from UVA to our grower (Kevin Engel, Cabin Hill Farms) on June 1, 2017.

The 6 hemp varieties used are as follows:
- Rocky Hemp - Rho-1 (Fiber)
- Colorado Star - CSO-1 (Fiber)
- Finola-O -1 (Fiber)
- Swift-1-V (Oil /Seed)
- Samuri-V (Oil /Seed)
- SKO-0 (Oil /Seed)

The seed provided to us was derived from the 2015/2016 seasons with stated germination rates of 70-74%. Prior to planting we analyzed germination rates in the laboratory and the amount of broken or damaged seed in the seed lots provided to us. For this analysis seeds were planted in moistened peat plugs or on sterile Whatmann filter paper. Scoring was done at four days and two weeks and these results indicated that some lines had germination rates below what was reported (in some cases approaching almost 40-50%). SKO-0 in particular was flagged as having low germination. We also noticed that there appeared to be some molding of seeds in some varieties. Whether this was due to insufficient drying during preparation or poor storage could not be determined. We used the recommended planting density provided by the supplier.

Planting Scheme and Method of Planting:

A simple planting scheme was used, in which each of the six test varieties was planted on an approximately 1 acre plot.

Seeds were drilled in 7” rows at a depth of ½ - ¾ inch using a Haybuster Multipurpose No-Till Drill (rented from the Department of Soil and Water Conservation in Hanover, VA). The seed drill was set to plant seeds at a rate of 30-50 lbs per acre per recommendation by the seed
supplier, and the seed bin on the drill was vacuumed out each time seed varieties were changed to prevent mixing.

**The first planting was done at Cabin Hill Farm on June 10, 2017.**

Germination was prompt, but low and spotty among some varieties, as predicted from the germination tests. Samuri-V and Finola-0-1 appeared to have better germination rates than the other varieties, but the field performance was somewhat disappointing. Under irrigation, growth of weed species in the soil bank quickly became problematic and the growth of the weeds provided severe competition to the young emerging hemp plants. This, coupled with the low, sporadic germination of some varieties, led us consider terminating the initial planting trial and replanting of seed at a higher density.

After ~4 weeks of growth, weeds, predominately pigweeds (*Amaranthus retroflexus* and *A. spinosus*), lambsquarters (*Chenopodium album*), various nettles and grasses and Palmer Amaranth (*Amaranthus palmeri*) were especially abundant. Weeds eventually overtopped the hemp at this site and the decision was made to terminate the trial. The Cabin Hill Farm site was disked under.

Due to a limited amount of seed and the lateness of the season, we also decided to forgo planting at Tree Hill Farms and to use the remaining seed to replant at Cabin Hill Farm and to plant at Morven Farms.

**Re-planting at Cabin Hill Farm occurred on July 10, 2017.**

Following termination of the initial planting, the field was disked and sat idle as weather and other demands on planting equipment needed to be accommodated. As in the earlier trial, seeds were drilled in 7” rows at a depth of $\frac{1}{2} - \frac{3}{4}$ inch using a Haybuster Multipurpose No-Till Drill and the drill was set to plant seeds at a rate of 50 lbs per acre per. Those varieties that were low and spotty were given increased planting rates.

Nonetheless, we still observed poor growth and it appeared that the germination rates were significantly lower. Two factors may have contributed to this. One factor is that we observed that some varieties had an issue with mold development, likely due to too high of moisture left in the seed after harvest. The other factor is that germination rate decline could have been due to storage since no extra precautions other than routine storage conditions were used.

As with the initial planting, under irrigation the weed seedlings provided severe competition to the hemp plants and weeds eventually overtopped the hemp at this site. Representative photographs of the fields are shown Figure 1 and it is evident that while there are some hemp, this is not at all suitable for further analysis of production.

Therefore, we were forced to make the unfortunate decision to once again terminate the trial in order not to severely cripple the fields with heavy seed banks for future plantings. The trial was terminated soon after August 6, 2017. The Cabin Hill site was disked under and prepared for planting of other crops.
Hemp Fields at Morven Farms.

Like at Cabin Hill Farms, a simple planting scheme was used, in which each of the six test varieties was planted on an approximately 1 acre plot.

Seeds were drilled in 7” rows at a depth of ½ - ¾ inch using a Haybuster Multipurpose No-Till Drill set to plant seeds at a rate of 50 lbs per acre. Since we anticipated issues with low germination of some varieties all remaining seed was used for planting.

Planting at Morven Farms occurred on July 13, 2017.

This was much later than we had hoped but we were able to get reasonable growth for 5 of the six varieties although some sections of the field were thinner than others. Just after planting there was an unusually hot dry spell with very little rainfall and both the hemp seedling and weed growth was slow. At about three weeks after planting there was a significant rain and the hemp began to take off. Since this time intermittent rain showers have allowed the hemp to become well established and 5/6 lines have shown significant growth (See Figure 2).

At the time of preparation of this report we are in the process of collecting data on the field performance of the varieties.

Phenological and Growth Parameters

Plant density, plant height, stalk diameter, fiber yield, and grain yield will be measured at the conclusion of the growing season. Since at the time of this report we are still in the process of evaluation, the data presented are not complete. On August 26 (44 days after planting) measurements were made at two well-spaced 1 square yard (yd²) locations within the variety plots. We collected data on plant height (mean and range) and total biomass. For biomass analysis, the plants in each sampling quadrant were hand pulled from the soil, all attached soil was removed from the roots and the fresh weight of the material determined. The plant material was then fast dried for a minimum of 2 h at 155°C in order to determine plant dry matter (Table 2). In some cases we excluded plants at the later time points that appeared to be the product of recent or delayed germinations since they were well off the mean.

Table 1. Varietal growth characteristics

<table>
<thead>
<tr>
<th>Variety</th>
<th>Plant Ht (in) 22 DAP</th>
<th>Plant Ht (in) 44 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho-1</td>
<td>4-6</td>
<td>72 (45-80)</td>
</tr>
<tr>
<td>CSO-1</td>
<td>8-10</td>
<td>40 (36-48)</td>
</tr>
<tr>
<td>Finola-O -1</td>
<td>6-10</td>
<td>52 (44-63)</td>
</tr>
<tr>
<td>Swift-1-V</td>
<td>6-10</td>
<td>36 (m) 48-60 (f)</td>
</tr>
<tr>
<td>Samurai-V</td>
<td>7-12</td>
<td>50 (44-72)</td>
</tr>
<tr>
<td>SKO-0</td>
<td>4-7</td>
<td>36 (22-50)</td>
</tr>
</tbody>
</table>

# DAP – days after planting; mean and range of plants in 1 yd² plots is given
Table 2. Varietal biomass production at Morven Farm

<table>
<thead>
<tr>
<th>Variety</th>
<th>Variety</th>
<th>FW* lbs/yd²</th>
<th>Est.¹ lb FW/A*</th>
<th>Est. Tons FW/A* (Green)</th>
<th>DW** lbs/yd²</th>
<th>Est. lb DW/A*</th>
<th>Est. Tons DW/A* (Straw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finola –O-1</td>
<td>S1</td>
<td>1.34</td>
<td>6485</td>
<td>3.8</td>
<td>0.40</td>
<td>1936</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>1.85</td>
<td>8954</td>
<td></td>
<td>0.51</td>
<td>2468</td>
<td></td>
</tr>
<tr>
<td>Samuri-V</td>
<td>S1</td>
<td>3.01</td>
<td>14,568</td>
<td>9.0</td>
<td>0.81</td>
<td>3920</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>4.49</td>
<td>21,731</td>
<td></td>
<td>0.51</td>
<td>2468</td>
<td></td>
</tr>
<tr>
<td>Rho -1</td>
<td>S1</td>
<td>2.44</td>
<td>11,809</td>
<td>9.1</td>
<td>1.20</td>
<td>5808</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>5.11</td>
<td>24,732</td>
<td></td>
<td>0.57</td>
<td>2759</td>
<td></td>
</tr>
<tr>
<td>CSO-1</td>
<td>S1</td>
<td>1.59</td>
<td>7,696</td>
<td>5.2</td>
<td>0.37</td>
<td>1791</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>2.70</td>
<td>13,068</td>
<td></td>
<td>0.54</td>
<td>2614</td>
<td></td>
</tr>
<tr>
<td>Swift -1-V</td>
<td>S1</td>
<td>1.56</td>
<td>7,550</td>
<td>3.7</td>
<td>0.39</td>
<td>1888</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>1.55</td>
<td>7,502</td>
<td></td>
<td>0.50</td>
<td>2420</td>
<td></td>
</tr>
<tr>
<td>SKO-0</td>
<td>S1</td>
<td>2.36</td>
<td>(11,422)</td>
<td>(5.7)</td>
<td>0.61</td>
<td>2952</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*FW (Fresh Weight); **DW (Dry Weight) or straw.

¹Biomass per acre was estimated by taking the value per yd² and multiplying by 4840 (yd² / A) and dividing by 2000 lb/ton.

Additional data on fresh and dry weight, plant height, stem diameter, seed production (grain number and grain mass), and biochemical characteristics (e.g., THC content, CBD/cannabinoid content, etc.) are not included here but will be determined at the end of the season.

Local VDACS personnel have been contacted and will come out to Morven Farm to sample the hemp seed and plant material for Δ-9 THC content. We anticipate that this will take place in mid- to late September about the time when 50% of the grain will be filled.

**Biological and other constraints:**

It was clear that competition from weed species can be a potentially severe problem using no-till agricultural approaches. We note that at Morven Farms, where no irrigation was supplied and plants relied on rainwater (which was limiting in late July-Early August) that the hemp growth was slowed initially but still outcompeted the weeds present. When rainfall did come (during the first weeks of August) the hemp plants took off and began to shade out the weeds as we would have expected. Samuri-V and Finola-O-1 did the best this season, followed by CSO-1 and Rho-1.
The major problems we encountered at Morven Farms were pigweeds, lambsquarters, Jimsonweed (Datura stramonium), foxtail (Setaria sp.) and other weeds were present in lesser amounts. Residual wheat from prior planting was also found among the rows.

**Objective 2. Laboratory-and greenhouse based studies to extend the genetic potential of hemp for agronomic and medicinal/nutritional purposes**

Greenhouse and laboratory studies are currently underway. This aspect of our work is primarily focused on two aspects: (1) best practices for the growth of high CBD strains under greenhouse conditions for the production of flowers for cannabinoid production and seed for propagation; and (2) development of expertise in traditional and molecular breeding to create new varieties. We currently have seven high CBD varieties growing in our research greenhouses at Gilmer Hall (Figure 3). These are varieties as follows:

- Otto II (2016)
- AC/DC (2016)
- Otto II X AC/DC (2016)
- Acota X Colorado Star (2016)
- Pewter River (2015)
- Special Sauce X AC/DC
- BoAx II (2016)

Plants are being grown to maturity, and pollen is being collected from male plants and stored for making self- and inter-varietal crosses. Seed will be collected and used for downstream greenhouse and field evaluation in the 2018 growing season.

We will also be collecting leaf tissues, floral parts, and seeds for measurement of total CBD levels and compositional differences among the various cannabinoids and terpenes present in the plant.

In the laboratory, we are focused on the development of *in vitro* culture conditions for the growth, regeneration, and vegetative propagation of elite lines of high CBD strains. We are also interested in developing new gene transfer (plant genetic engineering/transformation technologies) for hemp. At the present time little is known about the requirements for *in vitro* culture of hemp and even less information is available of the variability of responses among different hemp genotypes.

We have initiated basic studies to determine the response of the various germplasm to general *in vitro* culture conditions that test which plant growth medium works best and which balance of plant phytohormones are most effective in the establishment of callus, root, and shoot generation drive plant embryogenesis leading to regenerated growth. We are also determining the optimal experimental conditions for plant genetic transformation based upon the use of one of several gene transfer technologies: biolistic gene transfer, *Agrobacterium*-mediated gene transfer, and DNA-free genome editing with preassembled CRISPR-Cas9 ribonucleoproteins. The latter of
course will allow the production of modified hemp that is not considered GMO. The long term aim is the development of molecular genetic tools for Cannabis, with an eye towards the generation of hemp varieties that have unique agronomic characteristics or biochemical compositions of agricultural, nutritional, or medicinal value. In all cases the end goal is plants with low or no THC.

**Sub-Project: The Use of Industrial Hemp for Land Reclamation in Southwest Virginia**

Collaborators at UVA-Wise:
- Ryan Huish, PhD (Biology)
- Bob Van Gundy, PhD (Geology)
- Floyd Beckford, PhD (Chemistry)
- Adam Jones, Undergraduate in Environmental Science

This sub-project was carried out in collaboration with the University of Virginia-Wise and is focused on the development of industrial hemp cultivars that are capable of growing in the Southwest regions of the Commonwealth of Virginia where years of poor management practices and industrial mining have devastated soil quality and fertility. Thus, the aim of this research is a comparative analyses on the agronomic performance of various industrial hemp germplasms to determine which are best-suited for growth in the Southwest Region of the Commonwealth based on a range of outputs that deal with (i) ease of cultivation, growth rate, endurance to abiotic and biotic stressors throughout the growing season, (ii) quality of the final product (e.g. fiber content, seed quantity and quality, seed oil production, cannabinoid content); and (iii) capacity to sequester a range of heavy metals leading to phytoremediation. Inherent to this work is an analysis of the soil structure and composition prior to and following hemp growth in order to determine if there has been improvement that would indicate the potential for reclamation of land for use in hemp farming in the short term and broader agricultural use in the long term. Any identified cultivars will then be used in downstream breeding and selection programs intended to maximize the potential of industrial hemp in the region.

**Field site selection and preparation.**

Seven plots (designated 1-7) were selected on the UVA-Wise campus. The growing sites consist primarily of Kaymine series, a mixed loamy-skeletal soil, with hints of Fiveblock series present as well. Soil samples taken prior to planting revealed that the pH of the soil was well below the ideal range of 6.5 to 7.0, falling between 5.4 and 5.6. Liming the soil was not feasible due to the lack of time required for it to be incorporated into the soil.

Due to the presence of a very thin A-horizon of soil no greater than 2 inches, underlain by dirt composed of crushed rock containing no organic matter, diskimg or tilling the ground was deemed biologically detrimental. Instead, the fields were mowed, hayed, fertilized, and then mowed again the day before planting (see Figure 4).
The following organic fertilizers were applied on June 21, 2017 to each plot via a tractor-operated fertilizer spreader with the exception of feather meal which was applied using a walk-behind spreader:

1. Feather Meal 100 lbs per acre
2. Sodium Nitrate 100 lbs per acre
3. Potash 0-0-52 100 lbs per acre
4. Harmony 5-4-3 800 lbs per acre
5. Bone Meal 2-14-0 300 lbs per acre
6. Azomite 132 lbs per acre
7. Humic DG 40 lbs per acre

1/10th of each plot was left unfertilized as a control.

Seed Materials

The six varieties slated for planting in the trials at Cabin Hill Farm, Tree Hill Farm and Morven Farms, were also used at UVA-Wise along with a seventh variety specifically adapted for phytoremediation. The seeds were transferred from UVA to our collaborator/grower Dr. Ryan Huish, at UVa-Wise on June 3, 2017.

The hemp varieties used are as follows:
- Rocky Hemp - Rho-1 (Fiber)
- Colorado Star - CSO-1 (Fiber)
- Finola-O -1 (Fiber)
- Swift-1-V (Oil /Seed)
- Samuri-V (Oil /Seed)
- SKO-0 (Oil /Seed)
- Sterling Gold – StGo-1 (Oil/Seed, phytoremediation)

**The planting date at UVA-Wise was June 29, 2017.** A simple planting scheme was used, in which each of the seven varieties under evaluation was planted on an approximately 1 acre plot with the exception of Samurai-V which was divided into 2 separate plots totaling 1 acre (designated Plot 5.1 and 5.2). Seeds were drilled in 7” rows using a Great Plains 706nt no-till seed drill. The seed-drill was adjusted to the same settings used for Milo as the seeds are similar in size and shape to hemp seeds. The bin on the seed drill was vacuumed out each time seed varieties were changed to prevent mixing. The seed drill was set to plant seeds at a rate of 50lbs per acre for Plots 4, 5.1, 5.2, and 6, but was increased to 70 lbs per acre for the remaining plots because there were too many seeds left in the bin after all rows were completed in the first plots. Even after increasing seeding rate, there were still a large amount of seeds remaining in each bag. Seeds were drilled at ½” depth but the depth settings were adjusted multiple times to account for uneven ground, causing actual depth to fluctuate from surface level up to 2 inches in some places. The seed drill also left the cut open after seeding, as the soil was quite dry and the existing vegetation prevented the soil from moving back together behind the drill.
Phenological and Growth Parameters

Plant density, plant height, stalk diameter, fiber yield, and grain yield will be measured at the conclusion of the growing season. At the time of this report the field studies are still in progress and therefore we have not accumulated all of our data on plant growth and performance. Partial data are presented below.

Table 3. Varietal Performance at UVA-Wise

<table>
<thead>
<tr>
<th>Hemp Varieties:</th>
<th>Dates of emergence</th>
<th>Emergence (DAP)</th>
<th>Mean Plant Ht (in) 38 DAP</th>
<th>Date of first flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho-1</td>
<td>7/5/17</td>
<td>6</td>
<td>5 in</td>
<td>Week 8</td>
</tr>
<tr>
<td>CSO-1</td>
<td>7/5/17</td>
<td>6</td>
<td>10 in</td>
<td>Week 7</td>
</tr>
<tr>
<td>Finola-O-1</td>
<td>7/3/17</td>
<td>4</td>
<td>7-12 in</td>
<td>Week 6</td>
</tr>
<tr>
<td>Swift-1-V</td>
<td>7/3/17</td>
<td>4</td>
<td>10 in</td>
<td>Week 7</td>
</tr>
<tr>
<td>Samurai-V</td>
<td>7/3/17</td>
<td>4</td>
<td>10 in (5-27)</td>
<td>Week 8</td>
</tr>
<tr>
<td>SKO-0</td>
<td>Little to no germination</td>
<td>(unknown) 2 plants</td>
<td>4.5 in</td>
<td>n/a</td>
</tr>
<tr>
<td>StGo-1</td>
<td>7/4/17</td>
<td>5</td>
<td>4-14 in</td>
<td>Week 8</td>
</tr>
</tbody>
</table>

# DAP – days after planting

General Observations:

Limited rainfall and aggressive weed growth in the absence of management limit severely plant growth. Finola-O-1, Samuri-V, and Rho-1 are the varieties where we saw initially the highest surviving plant density under adverse conditions; StGo-1 and CSO-1 have the lowest density of visible plants at these point; and SKO-0 showed no appreciable germination and growth. With the coming of appreciable rainfall, Samuri-V (at 47 days after planting) continued to do well, with bushy plants that easily compete with surrounding vegetation. Finola-O-1, which seemed promising at first, has since declined due to the dryness and heat, along with significant grasshopper damage. Heat and inability to compete with the thriving grass resulted in the loss of nearly all Swift, CSO-1 and StGo-1 plants with the exception of several small (20’ X 30’2) patches of plants. While some varieties have plants of appreciable size (e.g., the largest individuals are found among Finola-O-1, Samuri-V, and CSO-1; these stand ~ 24 inches or more) the largest majority of the plants growing are very small.

No biomass or seed production data have been recorded to date, as no plants have been harvested at this time. Eventually, harvesting will be done by hand since the plants are so few and so small. The individuals that did survive may represent a very hardy gene pool that may do well on reclaimed mine land, given improved preparations and planning. These improvements could include raising the pH of the soil by applying lime months in advance, planting earlier during the wet season with a higher seeding rate, and tilling some plots for comparison. We would also like
to extend the research to include historical tobacco fields in the region, which should have better quality soil.

**Soil heavy metal analysis and metal phyto-sequestration analysis by hemp varieties:**

We completed the heavy metal analysis of soil samples (three per plot) on the sites at UVA-Wise. ICP-MS was used to measure aluminum, arsenic, cadmium, cobalt, iron, lead, manganese, nickel, and zinc. We are in the process of evaluating these metal levels and determining whether any are elevated relative to other soils. We plan to carry out a detailed analysis of the heavy metal contents in the roots, stems, leaves, flowers, and seeds of the different hemp cultivars at the end of the season.

**Lessons learned at UVA-Wise:**

Plants in the UVA-Wise fields were subject to strong insect herbivory, principally grasshoppers early on in the season and later Japanese beetles. Using no-till agriculture in which existing vegetation is not removed by significant herbicide burn-off or by diskng (deemed impractical in the low-topsoil environment near Wise) dicot weeds and grasses that predominate the existing vegetation recover quickly and outcompete the hemp seedlings. However, hemp plants that were able to establish themselves within areas of thick grass and vegetation where less bare ground is visible appear to be healthier than those plants established in the dry bare areas.

Since a major focus of this work is in phytoremediation and land reclamation we will perform an analysis of heavy metal content in the roots, stems, leaves, flowers, and seeds of the different hemp cultivars.
Figure 1. Hemp Field at Cabin Hill Farm (second planting) – August 7, 2017.
Shows fields that due to poor hemp seed germination are under severe competition from weeds.
Figure 2. Hemp Field at Morven Farms over the Season:

June 6, 2017 (unplanted field before bush-hogging and disking for seed bed preparation)

August 4, 2017 (Early plant growth – 22 days after planting)

August 11 2017 (29 days after planting; following first significant rainfall.)
August 22 2017 (40 days after planting)
August 26, 2017 (First plot sampling for biomass production; 44 days after planting) Shown is Samuri-V plot.
Figure 3. High CBD lines growing in UVA greenhouses for seed production and genetic studies, and (lower panels) hemp lines in cell culture for genetic transformation.
Figure 4. Hemp Field at UVa-Wise

Samuri-V plot as of week 8 (44 DAP). Hemp plants are still hardly distinguishable.

Hemp plants among the weeds (August 11, 2017; 42 days after planting)
Evaluating growth and productivity of several industrial hemp (*Cannabis sativa* L.) varieties in Virginia

Annual Report to
Virginia Department of Agriculture and Consumer Services

By

Maru Kipleting Kering, Ph.D.

Agricultural Research Station
Virginia State University
Petersburg, VA

September, 2017
Non-Technical Summary

During the 2017 production years, a total of twelve varieties of industrial hemp were grown at the Virginia State University Research and Demonstration Farm (Randolph Farm). Three studies were carried out; i) Variety trial; ii) Planting date study; iii) Nitrogen fertilizer study. Under the variety trial study, six varieties were grown for seed, five varieties for fiber, and one variety for both seed and fiber. Nitrogen fertility study involved one grain variety; Felina 32 and one fiber variety; Carmagnola, the same varieties used in 2016. Planting date study involved five planting dates carried out at approximately two week interval beginning mid-April through mid-June. Preliminary result indicates that early/mid-May is appropriate planting date. Also differences in varieties tolerances to early planting was observed. There is a huge pest problems that needs to be addressed and will include wild pest, mites and insect pest as well as seed destruction by wild birds

Major goals of the project

The project seeks to evaluate both fiber- and grain-type hemp varieties for establishments and growth performance in Virginia. The project seeks to determine; 1) Industrial hemp varieties that are suited to the southeast Virginia conditions; 2) The effect of nitrogen fertilizer on biomass, grain yield and grain quality; 3) Effect of planting date on growth and yield.

Expected Outcome

Outcome expected from this project includes; 1) Information on fresh/dry biomass of fiber- and seed yield of grain-type hemp varieties under different nitrogen fertilizer levels; 2) Information on growth and seed yield of different grain-type industrial hemp varieties at what would be considered optimal nutrient fertilizer levels, 3) impact of planting date on both fiber and grain type industrial hemp varieties

Accomplishment

During 2017, a total of 12 varieties were planted at the VSU Research and Demonstration (Randolph) Farm. Three studies: a) Variety trial; b) Planting date (PD) study; c) Nitrogen (N)
fertilizer study were carried out. In the variety trial a total of six varieties classified as grain-type were evaluated for grain yield. Five varieties were grown and evaluated for fiber. In addition one variety (Biedloreski) was evaluated for both grain and fiber. In the PD study, all five fiber-type and four selected grain-type varieties were evaluated. For nitrogen study, Felina 32, a grain-type variety and Carmagnola, a fiber-type variety were independently evaluated. For variety trial and nitrogen study, planting were planted on the 1st of June and 5th of June, respectively. For PD study, first planting was on the 14 April and approximately every two week thereafter with the fifth and last planting on June 14, 2017. All plots received P and K fertilizer based on soil analysis and as recommendation for a corn crop. Except for the N study, grain-type and fiber type varieties receive 200 kg N ha⁻¹ and 50 kg N ha⁻¹, respectively. For N fertilizer study, rate ranging from 0-100 kg N ha⁻¹ for fiber-type and 0-200 kg N ha⁻¹ for grain-type were applied. Similar seeding of rates and spacing of 22 kg ha⁻¹ and 38 cm (grain-type) and 67 kg ha⁻¹ and 19 cm (Fiber-type) as used in 2016 were used in 2017. There were observable differences in seedling emergence and plant density differed among varieties and planting dates. Soil moisture condition at planting and in days after affected emergence rates.

The crop showed good establishment and growth. The fiber-type variety was harvested at late flowering stage prior to seed maturity, and dry biomass determined.

**Crop Growth, Field Observations, and Data Collection**

In general there was poor seedling emergence and early crop growth for crop planted in mid-April. This is a period associated with a lot of rainfall and potential for cold temperatures especially during the night. Most likely germinated seed died out during days with extremely low night temperatures. Also, excessive moistures and flooded condition of some of the plots killed young emerging seedlings. However, those surviving plants went ahead to grow robustly produce good seed and fresh biomass yield (Figure 1a & 1b)

The crop planted in early May and mid-May showed betters emergence, a better plant density and was a more vigorous initial growth. However, there was a lot of rainfall during this period that resulted in excessive soil moisture and flooded sections of the plots. In these water logged patches, some seedling deaths were observed. The crop planted at these two dates grew more
vigorously and showed potential for better grain and fresh biomass yield (Figures 2a & 2b, 3a & 3b).

The crop planted in early-June (Figure 4a & 4b) and in mid-June (Figure 5a & 5b) were subject to a dry spelt and while seedling emergence was good, early crop growth was arrested and ended with shorter plants. Also as days shorten especially for the last two planting dates, reproductive stage set in earlier before appreciable amount of vegetative growth had been achieved. This late crop unlike those planted early were subject to excessive weed competition later during the summer months (Figure 6a & 6b), probably, a result of poor canopy development and poor soil cover. While seed and biomass yields are yet to be processed, it is most likely low.

In the N fertilizer study, there was a clear response to N fertilizer by the two varieties. In the Felina 32, lack of N led to yellowing of leaves and stunted growth (Figure 7a). Plants receiving N had better growth and robust and green leaves (Figure 7b). Similar result were observed in Carmagnola, the fiber-type variety.

There was significant infestation by insect pest and a whole array of insects were observed either feeding on or living under the crop foliage and other part (Figure 8). Also wild birds infestation especially at seed maturity was serious. There was also evidence of root rot especially young seedling in flood-prone plots. While no serious diseases were observe, some fungal molds were observed in some crops later in the season.

During harvesting, grain was obtained from a selected area within the middle rows and in fiber-type varieties, representative row length within the middle rows were harvested for dry biomass determination. In the grain variety, plants within a length of 3 m in the middle two rows were harvested, bagged and dried. Grain yield will be determined after it is shelled.
Figure. Industrial hemp grain-(a) and fiber-(b) crop planted in mid-April (1), early-May (2), mid-May (3), early-June (4) and mid-June (5) at Virginia State University Research and Demonstration Farm
Figure 6. Weed density in an early (6a) and a late planted industrial hemp grain crop.

Figure 7. Felina 32 grown at 0 kg N ha\textsuperscript{-1} (a), 100 kg N ha\textsuperscript{-1} (b) and 200 kg N ha\textsuperscript{-1} (c)
Figure 8. Some of the pest found infesting industrial hemp crop planted at Randolph Farm, VSU
Future Plans
In 2018, Virginia State University will continue varietal evaluation, PD and N fertilizer studies. We intend on continuing the same varieties in 2018 and we may also try to work with producers and other interested parts. Laboratory analysis of harvested material will be conducted as possible. From our observation, a comprehensive research work involving entomologist and crop pathologist is needed to focus on field pest infestation and diseases. Recommendations on adapted varieties, optimum fertilizer rates and planting dates will be made based on data collected over a three-four year period.

In addition to these efforts, we will continue to build relationships with others including private sector participants in anticipation of future collaborative work if and when licensing barriers are removed and industrial hemp production allowed in the Commonwealth of Virginia.
Appendix D

2017 Virginia Tech Hemp Trials  
VDACS Annual Report, 1 September 2017

Virginia Tech conducted research trials with industrial hemp for its second growing season in 2017. Dr. John Fike has remained the lead investigator and point of contact for studies conducted through Virginia Tech. Seed varieties for the 2017 growing season were imported from Canada and various countries in Europe.

Virginia Tech researchers planted hemp at 5 locations across the state in 2017: Blacksburg (Kentland Farm), Orange (Crop and Soil Environmental Sciences Research Center), Blackstone (Southern Piedmont Agricultural Research and Extension Center), Ruther Glen (private grower, Bill Pickett), and Gretna (private grower, Aubrey J. Nuckols). Sites at Orange and Blacksburg were used to conduct variety, planting date, herbicide tolerance, and cannabidiolic acid (CBDA) trials. A total of 20 varieties were grown and four planting date trials were incorporated for fiber and grain varieties. In Blackstone, Dr. Carol Wilkinson used a single variety (Felina 32) grown for multiple purposes (grain, fiber, and dual) to explore fertility management as well as the effects of different amounts of nitrogen on fiber and grain yield.

Table 1. Varieties tested by location in Virginia Tech hemp trials, 2017

<table>
<thead>
<tr>
<th>Location</th>
<th>Fiber</th>
<th>Grain</th>
<th>Fiber</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacksburg</td>
<td>Fibrinova</td>
<td>Glesia</td>
<td>Felina 32</td>
<td>Tygra</td>
</tr>
<tr>
<td>Orange</td>
<td>Compana E</td>
<td>Delores</td>
<td>Tygra</td>
<td>Felina 32</td>
</tr>
<tr>
<td></td>
<td>Canda</td>
<td>Biedloreski</td>
<td>Biedloreski</td>
<td>Ferimon</td>
</tr>
<tr>
<td></td>
<td>Wojko</td>
<td>Helena</td>
<td>Carmagnola</td>
<td>Biedloreski</td>
</tr>
<tr>
<td></td>
<td>Tygra</td>
<td>Joey</td>
<td>Canda</td>
<td>Helena</td>
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<tr>
<td></td>
<td>Fedora</td>
<td>Canda</td>
<td>Helena</td>
<td>Canda</td>
</tr>
</tbody>
</table>
Objectives for 2017 Industrial Hemp Trials

1) To measure plant parameters and biomass yield responses of hemp cultivars grown in various regions of Virginia.
2) To assess hemp injury tolerance from different herbicide applications.
3) To determine hemp yield response to different planting dates and rates.
4) To evaluate fertility effects on CBDA content in one variety (Carmagnola).
5) To survey insect pressures and their effect on crop injury and yield.

Variety Trials and Planting Date Study

Variety trials were repeated in 2017 in Blacksburg and Orange. Several new varieties were evaluated in the fiber and grain potential. Four planting dates (June 1st, June 14th, June 29th, July 13th) were chosen in Blacksburg to compare crop productivity and yield of several varieties in a separate study. Seeds were imported from Canada and various countries in Europe. The experimental design for the variety trial was a completely randomized design (CRD) and the design for the planting date study was a randomized complete block design (RCBD).

Fiber and grain variety trials were seeded in Orange on June 1st. Winter wheat was previously grown in the same area before hemp was planted. Leftover straw content from wheat harvest covered the field making it challenging for the hemp seeds to establish good contact with soil. Plots were sprayed with glyphosate at an application rate of 32 fl oz/ac (2.34 L/ha) and mowed initially in the middle of May to relieve this problem. Due to the coverage of the straw content and soil conditions, it was a collective decision to not plow the plots before planting - this was opposite of the tillage approach in the previous growing season. Urea fertilizer (46-0-0) was spread at rate of 200 lb/ac (178 kg/ha) for grain varieties.
and 60 lb/ac (53 kg/ha) for fiber varieties after seeding had occurred. The seeding rates for fiber and grain were 60 lb/ac (53 kg/ha) and 20 lb/ac (17.8 kg/ha), respectively. Fiber plots had 7.5 inch (19.05 cm) row to row spacing and grain plots were set to 15 inch (38.10 cm) row spacing. The dimensions for all plots were 5 ft x 16 ft (1.52m x 4.88m).

Fiber and grain variety trials were initially seeded in Blacksburg on June 2nd. The area, previously, had limited management and was mainly used for grass coverage for making bales of hay. Soil quality differed tremendously in sections of this area with some sections having more clay content versus others with silt-loam type soil. The grain variety trial had to be replanted on June 14th due to an undesirable first stand caused by planting issues, rough soil, and climate conditions. Urea fertilizer (46-0-0) was spread at rate of 200 lb/ac (178 kg/ha) for grain varieties and 60 lb/ac (53 kg/ha) for fiber varieties after seeding had occurred. The seeding rates for fiber and grain were 60 lb/ac (53 kg/ha) and 20 lb/ac (17.8 kg/ha). Fiber plots had 7 rows with 7.5 inch (19.05 cm) row to row spacing and grain plots had 4 rows with 15 inch (38.10 cm) row spacing. The dimensions for all plots were 5 ft x 16 ft (1.52m x 4.88m).

Plots were sprayed with glyphosate at an application rate of 32 fl oz/ac (2.34 L/ha), mowed, and plowed initially in the middle of May to relieve weed issues in the future. Plots for the planting date study were re-plowed at the time of seeding to provide suitable soil conditions for germination. Weeds have been a major concern in our grain plots shading out hemp and suppressing their vegetative stages. The row spacing and seeding rate of fiber lines has not allowed for many weeds to emerge. Johnsongrass, common ragweed, gypsum weed, crabgrass, and velvet leaf were the weeds scouted in the field where hemp productivity was low.

**Herbicide Tolerance**

This season, Virginia Tech incorporated a crop injury study from various herbicides with different modes of action. The area established for this study was under fiber and grain management with 7.5 inch (19.05 cm) and 15 inch (38.10 cm) row to row spacing, respectively, allowing for more weeds to emerge. Percentage crop injury and percentage chlorosis from herbicides were the primary ratings of interest for this study. Herbicide candidates were chosen from a greenhouse study prior to the field study. Pre-emergent herbicides were applied to the fiber managed area 24 hrs after planting. Post-emergent herbicides were applied when plants reached ~ 11 in. (27.94 cm) in height. Fiber biomass and grain yield will be collected at harvest using a wintersteiger small plot combine.

**CBDA Study**
In its first year, a CBDA (cannabidiolic acid) extraction study was incorporated into our 2017 trials following the announcement of the partnership between Synthonics Inc. and Virginia Tech. Synthonics Inc. is a specialty pharmaceutical company that focuses solely on metal coordinated pharmaceuticals. The company extracted cannabinoids from one medicinal variety (Carmagnola) in efforts to develop a purification process for cannabinoids of interest. Virginia Tech grew 140 plants in a greenhouse setting to ensure enough female plots were available to conduct the test. Plants were grown in a bark based medium in cone-tainers and received a nutrient fertilizer (24 - 8 - 16) at a rate of 60 lb/ac (53 kg/ha) to initiate emergence and growth. Once plants had reached a certain height between 5 to 7 in (12.70 cm to 17.78 cm), 45 plants were transplanted to the field plot where they would receive fertility, pruning, and combination treatments. A total of 7 plants in the field were males and had to be pulled, leaving 38 females to treat and test. Biomass content and CBDA % (by mass) were the ratings of interest for this study.

**Entomological Research**

After observing a lot of insect pests on hemp in the fall of 2016, entomological studies began in 2017. There were a range of insects present on plants of all varieties and planting dates, including stink bug, Japanese beetle, tarnished plant bug, corn earworm, and aphids. Research is occurring to determine the potential impact of insect pests upon hemp plants.
Two particular studies were conducted in Blacksburg in 2017. Insects with elevated populations in field hemp (Japanese beetle, tarnished plant bug, brown marmorated stink bug, and corn earworm) were chosen as study subjects. Insects were captured from the field and caged on seed heads of plants in varying densities (0, 5, and 10 insects for Japanese beetle, tarnished plant bug, and brown marmorated stink bug; 0, 3 small, or 3 large larvae for corn earworm). Final yield differences will be compared between caged plants containing insects and caged plants with no insects to assess amount of damage inflicted. In another study, field grown hemp was sprayed with a broad-spectrum insecticide on four dates at two week intervals. Insecticide treated hemp will be compared with untreated hemp to assess amount of damage inflicted on a larger scale. Results for both of these studies will consist of comparing wet weights (weight taken of plant material at time of harvest) to dry weights (weight taken after plant material has been allowed time to dry).