



UF/IFAS Industrial Hemp Pilot Project

Two-Year Report to Governor, President of the Senate, and Speaker of the House of Representatives

Authors

Dr. Zachary Brym, Assistant Professor of Agronomy & Lead Scientist

Jerry Fankhauser, Assistant Director of Florida Agricultural Experiment Station & Lead Oversight Manager

Overview

Industrial hemp is defined in Florida law as *Cannabis sativa* at or below 0.3 % total Delta-9 Tetrahydrocannabinol (THC) by dry weight and has been identified as a potentially valuable and impactful alternative crop for Florida. To support the future viability and sustainability of the emerging hemp industry, the University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) worked to become engaged in a preliminary assessment of the crop and cropping systems as this agricultural commodity moved toward commercialization in Florida. An industrial hemp pilot research project was approved by the Florida legislature (F.S. 1004.4473) in response to 7 U.S.C. s. 5940, with regulation of the pilot project established through FDACS 5B-57.013. The University of Florida was one of two land grant institutions in Florida specifically cited in the 2017 legislation as being authorized to engage in industrial hemp research and outreach. Those UF/IFAS pilot projects efforts, activities, and impacts are highlighted in this report.

This UF/IFAS Industrial Hemp Pilot Project report has been created and is being submitted as required by the following F.S. 1004.4473 language:

"A university that implements an industrial hemp pilot project shall submit a report to the Governor, the President of the Senate, and the Speaker of the House of Representatives on the status of its pilot project and any research related to the cultivation, harvesting, processing, and uses of industrial hemp. The report must be prepared and submitted within two years after the pilot project's creation."

Executive Summary

- UF/IFAS responded to the federal and state legislation to establish an industrial hemp pilot project with research and educational goals in support of hemp cultivation.
- The UF/IFAS Industrial Hemp Pilot Project obtained industry funding, University of Florida Board of Trustees approval, U.S. DEA Schedule 1 Registration, and Florida-FDACS-DPI permitting for project initiation in 2019.
- UF/IFAS hemp resources are available online at: <https://programs.ifas.ufl.edu/hemp/>
- Hemp may indeed be a viable agricultural commodity for Florida stakeholders with caution for economic and environmental challenges.
- UF/IFAS is prepared to continue the research and educational efforts with hemp.

With the passage of Senate Bill 1726 in 2017, leadership and researchers within UF/IFAS immediately began discussions to structure a hemp pilot project. The Office of the Dean for Research and the Department of Agronomy within UF/IFAS led the effort to organize a core team that included expertise in crop growth and development, pest mitigation, economics, and plant invasiveness. This team worked with UF/IFAS administration to produce a pilot project plan that was soon presented to interested parties within the hemp industry in Florida. This plan included efforts to identify hemp germplasm appropriate for Florida's diverse environmental and agronomic conditions, design and better understand cropping and controlled growth systems to serve a diverse range of hemp industries in Florida's various agricultural environments, and to assess and mitigate the invasion risk of industrial hemp.

Per statute, industry funding was required and once secured, UF/IFAS leadership took the pilot project plan and industry sponsorship to the Board of Trustees at the University of Florida for approval (see Appendix A for a listing of sponsors). Once approved, the UF/IFAS hemp team continued planning efforts while awaiting 2018 FDACS rulemaking needed for the commencement of industrial hemp pilot projects. Included in these planning efforts was the recruitment of both graduate students and post doctoral expertise (see Appendix B). In March of 2019, FDACS-Division of Plant Industry (DPI) issued the first research permit in the State of Florida for research with industrial hemp to UF/IFAS. This permit was for the DEA-approved industrial hemp seed intake site. To date, UF/IFAS has been issued 48 planting permits with 46 remaining active (see Appendix C). The UF/IFAS Industrial Hemp Pilot Project also obtained Drug Enforcement Administration (DEA) Schedule 1 registration for research with low THC *Cannabis sativa*. That registration (RU0537069) was secured in May of 2019 and remains active (see Appendix D).

For more information visit programs.ifas.ufl.edu/hemp

Research and education efforts for stakeholders in Florida commenced in the spring of 2019 with the UF/IFAS hemp team establishing multiple outdoor field sites under hemp cultivation and worked with UF/IFAS Extension and Communications to conduct workshops and generate educational materials. Fact sheets and other research information obtained across the pilot project are available at the project's website (Link: <https://programs.ifas.ufl.edu/hemp/>) and at the UF/IFAS Electronic Data Information Source (EDIS) online site, including a report on hemp [fertilizer guidelines](#). In December 2019, UF/IFAS leadership again sought approval from the Board of Trustees at the University of Florida to expand Industrial Hemp Pilot Project

efforts including qualifying project partners as allowed by Senate Bill 1726 with anticipated commercialization in 2020. These partners included family farms and ranches and industry in Florida.

Educational efforts commenced early on and included the development of a core team within UF/IFAS Research, Extension, and administration supporting initial workshops and field day efforts. Workshops in 2018 and 2019 included 265 and 600 participants statewide, respectively. In early 2021, the pilot project launched an online virtual workshop, which has been well attended. The workshop will remain available until November 1, 2021. Course viewership to date (as of 6.7.2021) is as follows:

Title	Presenter	Views (as of 6/7/2021)
"A Welcome to the UF/IFAS Hemp Online Shortcourse"	Zack Brym	117 views
"Impact of Plant Diversity on Yield and Cannabinoid Content of Industrial Hemp"	Sarah Benevenue	72 views
"UF/IFAS & Syngenta Public Private Sector Partnership in Industrial Hemp"	Heather Kalamani, Graduate Student Project Team	65 views
"Essential Oil Hemp Research Program at the UF/IFAS Mid Florida Research and Education Center"	Steve Anderson	60 views
"Hemp Yield and Cannabinoid Response to Nitrogen Rates in South Florida"	Luis Monserrate	60 views
"Evaluation of Industrial Hemp Varieties for Essential Oil Production in Florida"	Josh Freeman	58 views
"Photoperiodism of CBD and Fiber Hemp"	Mengzi Zhang	55 views
"Effect of Nutritional Supplementation of Hemp Essential Oil"	Luis Monserrate	51 views
"First Season Challenges with Hemp Production in Northwest Florida"	De Broughton	51 views
"Identification of Hemp Varieties for Muck and Sand Soils in Central South Florida"	Hardev Sandhu	50 views
"Establishing High CBD Hemp Cultivars in Tissue Culture"	Angelika Altpeter	41 views
"Insights to Building a Hemp Industry in Florida"	Trent Blare	39 views
"Developing a New Agent Specialist Partnership for Extension"	Christine Kelly-Begazo	35 views
"From Project to Program Growing Hemp Efforts at UF/IFAS"	Zack Brym	14 views
"Introduction to the Hemp Virtual Workshop"	Ajit Williams	4 views

UF/IFAS Industrial Hemp Pilot Project - Specific Results and Observations

- Variety trials across Florida show some promising hemp genetics for grain and flower production. Date-of-planting, effective pest control, and other management aspects with this crop are important.
- Many available hemp genetics planted in variety trials in 2019 and 2020 exceeded the 0.3% total THC threshold allowable, including the majority of genetics for flower production (i.e., CBD oil). This is problematic for Florida

growers given the federal and state consequences of a crop that exceeds the total THC limit.

- Caution is warranted for hemp escape from cultivation and possible invasiveness. Industrial hemp has been shown to be able to establish outside of cultivation in Florida with ideal conditions including high seed dispersal, highly disturbed soils, and open habitats.
- On-farm and industry-led studies via Qualified Project Partners (QPPs) with UF/IFAS are leading to direct industry engagement and targeted information regarding industrial hemp economics and overall production risks (see Appendix E).

- UF/IFAS Extension hemp core team was established and trained to include 15 agents and specialists available for engagement with stakeholders and educational programming throughout the state (see Appendix F).
- UF/IFAS leadership worked with hemp team faculty at the Mid-Florida Research and Education Center-Apopka and Qualified Project Partner, Roseville Farms, to develop and pilot project cultivar approval program for high cannabinoid clonal propagules (Link: <https://programs.ifas.ufl.edu/hemp/approval-program/>). Per state statute for pilot projects, UF/IFAS-approved genetics can be legally sold in the State of Florida. To date, UF/IFAS has approved 21 cultivars from industry in Florida and beyond.
- Initial stakeholder interest was more focused on high cannabinoid hemp production (e.g., CBD and CBG), but more recent UF/IFAS hemp team interaction with growers, those in industry, and FDACS leadership suggest that there may soon be hemp fiber industry development in Florida.

Communications Efforts

UF/IFAS researchers collaborated with the UF/IFAS communications team to ensure consistent, high quality communications and information was available for all interested parties. Materials created included videos, mid-project reports, newsletter, FAQs on hemp, hemp growing, hemp research and more.

In addition to creating the pilot program website at <https://programs.ifas.ufl.edu/hemp/>, the public relations

team assisted in writing and placing updates about the program in stakeholder and agriculture outlets as well as general media, managing media inquiries, and supporting the research team. Over the course of the 2-year pilot project effort, more than 40 stories were placed in the media, many of which can be found here: <https://programs.ifas.ufl.edu/hemp/news/#>.

Metrics

- Pageviews <https://programs.ifas.ufl.edu/hemp/> May 1, 2019 – May 31, 2021: 69,017
- Video views: 985 views for Hemp Workshop videos + 566 click events on uploaded Hemp presentations = 1,551 total views.
- Hemp Pilot Project Newsletter currently has 1,825 subscribers.
- Attendance at UF/IFAS Industrial Hemp Virtual Workshops since launch on 1/11/2021: 150 enrolled

Summary

In summary, the UF/IFAS Industrial Hemp Pilot Project team efforts over the past two years show aspects of hemp cultivation that could lead to a viable agricultural commodity for Florida stakeholders, but more research and funding for such efforts is needed (see Appendix G for examples of publications, reports, and presentations to date). The UF/IFAS Hemp Program is well positioned to continue the research to generate new knowledge and develop educational programs to effectively communicate to those seeking to grow, process, or use industrial hemp.

UF/IFAS Industrial Hemp Pilot Project – 2019 FDACS-approved Research Plan

Plan Overview

Industrial hemp (*Cannabis sativa*) has been identified as a potentially valuable and impactful alternative crop for Florida. To support the future viability and sustainability of a hemp industry, preliminary assessment of the crop and cropping systems must be established prior to commercialization. An industrial hemp pilot research project was approved by the Florida legislature (F.S. 1004.4473) in response to 7 U.S.C. s. 5940, with regulation of the pilot project established through the Florida Department of Agriculture and Consumer Services (FDACS)

5B-57.013. The purpose of the UF/IFAS Industrial Hemp Pilot Project is to identify hemp germplasm appropriate for Florida's diverse environmental and agronomic conditions, to develop cropping systems that serve a diverse range

of hemp industries, and to assess and mitigate hemp invasion risk.

Given the potential opportunities and challenges, a preliminary assessment by the UF/IFAS Industrial Hemp Pilot Project will be conducted to support the future commercialization of industrial hemp. To ensure a profitable and sustainable hemp industry in Florida, the pilot project will address the following overarching questions:

- How will hemp grow and reproduce on farms and in natural areas of Florida?
- What are the best hemp cultivars/varieties for Florida?
- How can existing farming equipment and operations be adapted to hemp production?
- What are the economic impacts of the pilot project?

Goals and Objectives

The preliminary assessment is organized as a multi-site collaborative experiment at various UF/IFAS research locations, guided by a UF/IFAS Department of Agronomy faculty team and strategic research and industry partners. The overall goal of the UF/IFAS research team is to help develop and support a profitable and sustainable hemp industry in Florida by addressing critical agronomic challenges. This proposal lays out short-term goals for the two-year pilot period, which is intended to motivate and inform future research programs, on-farm trials, and integration with the prospective processing industry. Following the pilot project, UF/IFAS is mandated to provide a report to the Florida legislature that describes the best available information for cultivation, harvesting, processing, and economic impact of industrial hemp generated by the pilot project. As such, the proposed project broadly aims to address these aspects of hemp production for a fair assessment of industrial hemp agronomic, economic, and environmental feasibility. The expected short-term outcomes of the project are to identify hemp varieties suitable for Florida, develop potential hemp cropping systems for commercial production, and assess hemp invasion risk. The specific objectives for each proposed outcome are as follows:

1. Identify hemp varieties suitable for planting in Florida's various environments.

Hemp varieties will be assessed for plant growth, health, and production with a focus on resilience to potential environmental, ecological, and economic threats. Variety trials will be established to identify a viable germplasm and to test planting date.

2. Develop hemp management practices and cropping systems suitable for Florida.

Hemp cropping systems will be designed for raw material production suitable to current farming and anticipated processing industries. The best available varieties will be integrated with continually updated cropping system recommendations to meet the needs of the prospective processing and market goals. Cropping system trials will test management practices and evaluate the economic break-even point.

3. Assess hemp invasion risk in Florida's natural and built environments.

Hemp invasion risk will be assessed for the various regions and environmental conditions anticipated for hemp production. The invasion risk assessment will be integrated with variety identification and cropping system development to mitigate the risk of hemp production.

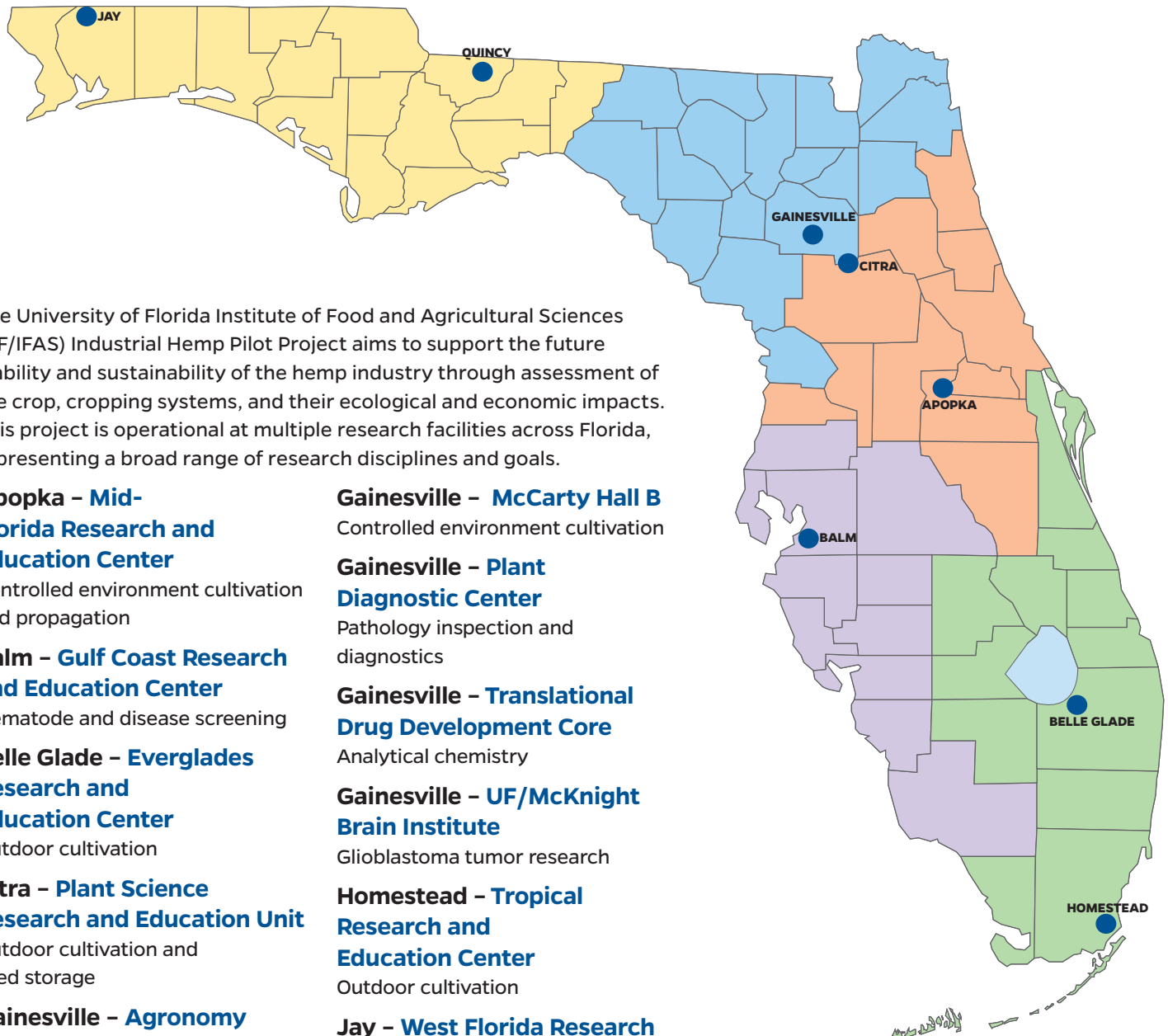
Initial Pilot Project Faculty and Staff

Initial UF/IFAS Faculty and Staff:

- Dr. Zachary Brym, Agronomy, Assistant Professor, TREC & State Research Coordinator
- Dr. Michael Mulvaney, Agronomy, Assistant Professor, WFREC Site Coordinator
- Dr. Josh Freeman, Horticulture Science, Associate Professor, NFREC Site Coordinator
- Dr. John Erickson, Agronomy, Associate Professor, AFRU Site Coordinator
- Dr. S. Luke Flory, Agronomy, Associate Professor, Bivens Arm Site Coordinator
- Dr. Edward Evans, Food and Resource Economics, Professor, TREC, Economic Analyst
- Dr. Hardev Sandhu, Agronomy, Assistant Professor, EREC Site Coordinator
- Dr. Rob Gilbert, Dean for Research - UF/IFAS Administration
- Jerry Fankhauser, Lead Oversight Manager - Florida Ag. Experiment Station-UF/IFAS

UF/IFAS Industrial Hemp Pilot Project

Permitted University Research Locations



The University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Industrial Hemp Pilot Project aims to support the future viability and sustainability of the hemp industry through assessment of the crop, cropping systems, and their ecological and economic impacts. This project is operational at multiple research facilities across Florida, representing a broad range of research disciplines and goals.

Apopka – Mid-Florida Research and Education Center

Controlled environment cultivation and propagation

Balm – Gulf Coast Research and Education Center

Nematode and disease screening

Belle Glade – Everglades Research and Education Center

Outdoor cultivation

Citra – Plant Science Research and Education Unit

Outdoor cultivation and seed storage

Gainesville – Agronomy Research Laboratories and Greenhouses

Controlled environment cultivation

Gainesville – Bivens Arm

Invasion risk experiments

Gainesville – McCarty Hall B

Controlled environment cultivation

Gainesville – Plant Diagnostic Center

Pathology inspection and diagnostics

Gainesville – Translational Drug Development Core

Analytical chemistry

Gainesville – UF/McKnight Brain Institute

Glioblastoma tumor research

Homestead – Tropical Research and Education Center

Outdoor cultivation

Jay – West Florida Research and Education Center

Outdoor cultivation

Quincy – North Florida Research and Education Center

Outdoor cultivation

Appendix A: UF/IFAS Industrial Hemp Pilot Project & Program Sponsors

Industrial Hemp Pilot Project (\$950,000 - \$90,000)

- Green Roads
- FDACS-Office of Agricultural Water Policy
- U.S. Sugar Corporation/Lykes Brothers, Inc.

Industrial Hemp Endowment Fund (\$90,000 - \$30,000)

- Fortin Foundation of Florida
- Roseville Farms
- Syngenta Flowers
- Florida Hemp Trade and Retail Association
- Star Manufacturing

Industrial Hemp Endowment Fund (\$15,000 and less)

- South Tip, Inc.
- Big Water Hemp
- J. Clayton Pruitt Family
- Enrique Yanes
- Kathryn Arnold
- Tater Farms
- Rolando Clavijo
- Diamond R Fertilizer
- Florida Hemp Conference
- Scott Prospect
- Dade County Farm Bureau
- Michelle Beasley & Robert Beasley
- EarthCorp Foundation
- Gail Stanberry
- Ralph Dominguez
- Jeffrey Adair
- Kayne Stewart
- Willie Patterson

Hemp Seed, Plants, and other In-kind Donations

- ANO CBD
- Big Water Hemp
- Eastern Plains Hemp
- Green Point Research
- Green Roads
- HM Health
- Roseville Farms
- U.S. Sugar Corporation
- Syngenta Flowers
- ING Hemp
- Kayagene
- New West Genetics
- Phylos
- Pure Beauty Farms
- Tahnja

2018 Workshop Series (\$1,000 - \$100)

- Hailey's Hemp Company
- Farmhouse Tomatoes
- Nature Coast Cannabis Enterprises
- Impact Landscape & Irrigation
- Pura Hemp
- Mari J Pharmaceuticals, Inc.
- Timothy F Stanfield, Attorney at Law

2019 Workshop Series (\$900 - \$300)

- The Florida Hemp Council
- TriEst Ag Group, Inc.
- Agromillora
- American Organic Food Company
- Canna Care Wellness
- Crosby and Associates
- Dean Mead Attorneys at Law
- Florida Foundation Seed Producers, Inc.
- Functional Food Systems, Inc.
- Intergro
- Jushi
- Mr. Cannabis Law
- Nature Coast Cannabis Enterprises
- Rose of Sharon Nursery, Inc.

Appendix B: Post Doctorates and Graduate Students involved in UF/IFAS Industrial Hemp Pilot Project

- Rui Yang, Post-doc
- William Wadlington, Post-doc
- Steven Anderson, Post-doc
- Susan Canavan, Post-doc
- Maryjo Valle, MS '21
- Luis Monserrate, MS '21
- Jacqueline Coburn, MS '21
- Sarah Benevenute, MS '21
- Tamara Serrano, MS *in progress*
- Navdeep Kaur, MS *in progress*
- Yogendra Upadhyaya, PhD *in progress*
- Saroop Sandhu, PhD *in progress*

Appendix C: FDACS-DPI Industrial Hemp Planting Permits

1. UF/IFAS Plant Science Research & Education Unit-Citra (Permit #001) – **Active**
Contact: Jim Boyer
2. UF/IFAS Mid-Florida Research & Education Center-Apopka - (Permit #002) – **Active**
Contact: Dr. Brian Pearson
3. UF/IFAS Bivens Arm Research Station-Gainesville (Permit #003) – **Active**
Contact: Dr. Luke Flory
4. UF/IFAS McCarty Hall-B-Gainesville (Permit #004) – **Active**
Contact: Dr. Luke Flory
5. UF/IFAS West Florida Research & Education Center-Jay (Permit #005) – **Active**
Contact: Dr. Mike Mulvaney
6. UF/IFAS Range Cattle Research & Education Center-Ona (Permit #006) – **Not Active**
Contact: Dr. Brent Sellers
7. UF/IFAS Everglades Research & Education Center-Belle Glade (Permit #007) – **Active**
Contact: Dr. Hardev Sandhu
8. UF/IFAS Gulf Coast Research & Education Center-Wimauma (Permit #008) – **Active**
Contact: Dr. Johan Desaegeer/Dr. Natalia Peres
9. UF/IFAS Agronomy Forage Research Unit-Hague (Permit #009) – **Active**
Contact: Dr. Diane Rowland
10. UF/IFAS Tropical Research & Education Center-Homestead (Permit #010) – **Active**
Contact: Dr. Zack Brym
11. UF/IFAS North Florida Research & Education Center-Quincy (Permit #011) – **Active**
Contact: Dr. Josh Freeman
12. UF/IFAS Plant Diagnostic Center-Gainesville (Permit #012) – **Active**
Contact: Dr. Carrie Harmon
13. UF/IFAS Agronomy Genetics & Physiology Labs/Greenhouses-Gainesville (Permit #020) – **Active**
Contact: Dr. Ali Babar
14. UF Translational Drug Development Core-Gainesville (Permit #027) – **Active**
Contact: Dr. Christopher McCurdy
15. UF/IFAS McCarty Hall-D-Greenhouses & Labs-Gainesville (Permit #033) – **Active**
Contact: Dr. Fredy Altpeter
16. Syngenta Flowers, LLC-Qualified Project Partner-Alva (Permit #034) – **Not Active**
Contact: N/A
17. USSC-Research Laboratory-Qualified Project Partner-Clewiston (Permit #037) – **Active**
Contact: Mike Irey
18. USSC-Townsite Farm-Qualified Project Partner-Hendry County (Permit #038) – **Active**
Contact: Mike Irey
19. USSC-Rita Farm-Qualified Project Partner-Palm Beach County (Permit #039) – **Active**
Contact: Mike Irey
20. USSC-Rucks Citrus Nursery-Qualified Project Partner-Frostproof (Permit #040) – **Active**
Contact: Mike Irey
21. Highland Hemp Farms-Basinger-Coon Island-Qualified Project Partner (Permit #041) – **Active**
Contact: Mike Irey
22. Highland Hemp Farms-Grove 3-Qualified Project Partner-Highlands County (Permit #042) – **Active**
Contact: Mike Irey
23. USSC-Greenhouse-Qualified Project Partner-Clewiston (Permit #043) – **Active**
Contact: Mike Irey
24. Highland Hemp Farms-Brighton Grove 7-Qualified Project Partner -(Permit #044) – **Active**
Contact: Mike Irey
25. Roseville Farms, LC-Buildings 7&10-Qualified Project Partner-Apopka (Permit #045) – **Active**
Contact: David Raab
26. Florida Panhandle Hemp-On-Farm- Qualified Project Partner-Overstreet (Permit #057) – **Active**
Contact: Kathryn Arnold
27. SouthTip-On-Farm- Qualified Project Partner-Homestead (Permit #058) – **Active**
Contact: Sal Finochiarro
28. CIGN LLC-On-Farm- Qualified Project Partner-Miami (Permit #059) – **Active**
Contact: Michael Feldenkrais
29. V&B Farms LLC-On-Farm- Qualified Project Partner-Homestead (Permit #060) – **Active**
Contact: Tommy Vick
30. LNB Groves-On-Farm- Qualified Project Partner-Homestead (Permit #061) – **Active**
Contact: Marc Ellenby

31. Ancient City Hemp-On-Farm- Qualified Project Partner- Hastings (Permit #064) – **Active**
Contact: Jeff Thomas
32. Bar 4J Bar Ranch-On-Farm- *Qualified Project Partner-* Sidell (Permit #065) – **Active**
Contact: Allison Flint
33. Bethel Farms-On-Farm- Qualified Project Partner- Arcadia (Permit #066) – **Active**
Contact: Jonathan Brown
34. CSX Ag-Good Earth Farm-On-Farm- Qualified Project Partner-East Palatka (Permit #067 – **Active**
Contact: Sebastien Gros
35. Florida Hybrid Solutions-On-Farm- Qualified Project Partner-Vero Beach (Permit #068) – **Active**
Contact: Carlos Hayden
36. PharmaSeed FL-On-Farm- Qualified Project Partner- Apopka (Permit #069) – **Active**
Contact: Albert Garcia
37. Pure Beauty Farms-On-Farm- Qualified Project Partner- Miami (Permit #070) – **Active**
Contact: Pedro DeMorejon
38. C&B Farms-On-Farm- Qualified Project Partner- Clewiston (Permit #071) – **Active**
Contact: Charles Obern
39. VersatiliTree Farms-On-Farm- Qualified Project Partner- Okeechobee (Permit #072) – **Active**
Contact: Scheril Murray Powell
40. Speedling Inc-On-Farm- Qualified Project Partner- Ruskin (Permit #073) – **Active**
Contact: Mark Worley
41. Fortis Genetics-On-Farm- Qualified Project Partner- Vero Beach (Permit #076) – **Active**
Contact: Bruce Vanaman
42. Price Creek Cattle Co-On-Farm- Qualified Project Partner-Lake City (Permit #077) – **Active**
Contact: Matt Dicks
43. Brookins Farms-On-Farm- Qualified Project Partner- Chiefland (Permit #078) – **Active**
Contact: Loran Brookins
44. UF-McKnight Brain Institute/Cancer Center & Genetics Center-Gainesville (Permit #079) – **Active**
Contact: Dr. Brent Reynolds
45. UF/IFAS Environmental Hort. Greenhouses-Ornamental Labs-Gainesville (Permit #086) – **Active**
Contact: Dr. Wagner Vendrame
46. UF/IFAS Southwest Florida Research & Education Center-Immokalee (Permit #091) – **Active**
Contact: Dr. Ute Albrecht
47. UF/IFAS Hastings Agricultural Extension Center-Cowpen Branch (Permit #092) – **Active**
Contact: Christian Christensen
48. UF/IFAS Hastings Agricultural Extension Center- Downtown (Permit #093) – **Active**
Contact: Christian Christensen

Date: June 2021

Appendix D: UF/IFAS Industrial Hemp Pilot Project's DEA Schedule 1 Registration



10000513.2/000824-1/1-0

1-3 UNIVERSITY OF FLORIDA, AGRONOMY DEPTME
G040D MCCARTY HALL
PO BOX 110200
GAINESVILLE, FL 32611-0000



DEA REGISTRATION NUMBER	THIS REGISTRATION EXPIRES	FEE PAID
RU0537069	05-31-2022	FEE EXEMPT
SCHEDULES	BUSINESS ACTIVITY	ISSUE DATE
1	RESEARCHER (I)	05-20-2021
UNIVERSITY OF FLORIDA, AGRONOMY DEPTME PLANT SCIENCE RESEARCH & EDUCATION 2556 W HIGHWAY 318 BLDG 7512 CITRA, FL 32113-2132		

CONTROLLED SUBSTANCE REGISTRATION CERTIFICATE
UNITED STATES DEPARTMENT OF JUSTICE
DRUG ENFORCEMENT ADMINISTRATION
WASHINGTON D.C. 20537

Restricted to Government personnel for official duties only.

Sections 304 and 1008 (21 USC 824 and 958) of the Controlled Substances Act of 1970, as amended, provide that the Attorney General may revoke or suspend a registration to manufacture, distribute, dispense, import or export a controlled substance.

THIS CERTIFICATE IS NOT TRANSFERABLE ON CHANGE OF OWNERSHIP, CONTROL, LOCATION, OR BUSINESS ACTIVITY, AND IT IS NOT VALID AFTER THE EXPIRATION DATE.

Form DEA-223 (9/2016)

CONTROLLED SUBSTANCE REGISTRATION CERTIFICATE UNITED STATES DEPARTMENT OF JUSTICE DRUG ENFORCEMENT ADMINISTRATION WASHINGTON D.C. 20537		
DEA REGISTRATION NUMBER	THIS REGISTRATION EXPIRES	FEE PAID
RU0537069	05-31-2022	FEE EXEMPT
SCHEDULES	BUSINESS ACTIVITY	ISSUE DATE
1	RESEARCHER (I)	05-20-2021
UNIVERSITY OF FLORIDA, AGRONOMY DEPTME PLANT SCIENCE RESEARCH & EDUCATION 2556 W HIGHWAY 318 BLDG 7512 CITRA, FL 32113-2132		

Restricted to Government personnel for official duties only.

Sections 304 and 1008 (21 USC 824 and 958) of the Controlled Substances Act of 1970, as amended, provide that the Attorney General may revoke or suspend a registration to manufacture, distribute, dispense, import or export a controlled substance.

THIS CERTIFICATE IS NOT TRANSFERABLE ON CHANGE OF OWNERSHIP, CONTROL, LOCATION, OR BUSINESS ACTIVITY, AND IT IS NOT VALID AFTER THE EXPIRATION DATE.

CENTER FOR PUBLIC ISSUES EDUCATION

IN AGRICULTURE AND NATURAL RESOURCES

Executive Summary Report

Experiences of On-Farm Trial Partners

Developed by Shelli D. Rampold for Zachary Brym

Executive Summary

On-Farm Trial Partner Experience

Focus Group Summary

June 7, 2021

Background

Focus groups were conducted with on-farm trial partners of the UF/IFAS industrial hemp pilot project. These focus groups were held to gain feedback from partners regarding their experiences as part of the project, included overall experiences, what worked or did not work, and to identify recommendations for future practice with trial partners.

Key Findings

The following section includes key findings pertaining to on-farm trial partners' (a) challenges experienced in growing hemp; (b) recommendations for future growing practices; (c) perceptions of the UF/IFAS pilot program's best characteristics and areas for improvement; (d) experiences with the UF/IFAS team leadership; and (e) other future concerns or considerations for the UF/IFAS hemp program.

Growing Experiences

First, farmer participants were asked about their experiences growing hemp as part of the UF/IFAS hemp pilot program.

Growing Challenges

- Overall, farmer participants reported mixed results for strains. Some reported more success with Cherry Wine, while others reported more success with Maverick.
- Key growing challenges related to weather included:
 - Heavy rain and resulting crop loss
 - Heat and trouble keeping soil moist enough to germinate successfully on scale
 - Humidity
 - Hurricanes
- Key growing challenges related to pests or weeds included:
 - Pest pressure from caterpillars, grasshoppers, and ants
 - Being unable to fight the weed pressure with flat beds without plastic and recommended raised beds and plastic coverings.
 - Fungal pressure
- Other key challenges included:
 - Fertilization issues in sandy soil
 - Stunting at germination
 - Poor stand for some participants

- Late planting (and associated quick flowering, short days, the photoperiod, etc.).
- Direct seed was a challenge for some, and the option of half growing to become a transplant was recommended as a potential option for the future (especially for those without a nursery license).
 - Trouble with direct seed, along with heat and humidity, was particularly a challenge for those in South Florida
 - Other growers from South Florida also echoed that summer may not be the best planting time for them
- Team setbacks from the University with COVID-19 and outcomes of pandemic changes
- Collaboration and communication for getting supplies – especially for those not in “big agriculture” areas

Growing Recommendations

- Farmer participants described several recommendations for things they did or may do differently if they were to grow hemp again. Such recommendations included the following:
 - Raised beds were recommended by many farmers as the “best way to go.”
 - Using plastic coverings on beds
 - Planting earlier (e.g., March)
 - Using container processing environment that can move from farm to farm so it can be processed through the university and pushed through to efficacy studies

Pilot Program Experiences

Next, farmer participants were asked questions pertaining to their experiences with the format, structure, and leadership of the UF/IFAS hemp pilot program. Specifically, they were asked to describe what worked for them, what could use improvement, their experiences interacting with the leadership members of the program, and any recommendations they may have for the program in the future.

Pilot Program Best Qualities

- Providing seeds was perceived as extremely useful among farmer participants. Some noted they may not have participated in the program had the seeds not been provided for them.
- Participants noted that program leaders were available and attentive to their needs and questions.
- Extension agents were identified as a key component to their success and positive experience.
- Application process was straightforward, clear, and quick
- Elimination of bond requirement was good, especially for minority and small farmers
- Overall really supportive environment
- Start-up costs were not a barrier to most participants, and many found it easy to incorporate hemp into what they were already doing with the resources they already had.
 - Again, the donated seeds played a large and positive role

Pilot Program Challenges

- Some participants noted difficulty in staying in touch with UF/IFAS team due to circumstances surrounding COVID-19.

- Many participants were understanding of the challenges, with one participant noting that “we are all in the same boat in an unprecedented time.”
- Other participants describe difficulty with the short timeline or “time-crunch” in trying to meet all the growing deadlines and red tape.
- One participant mentioned a breakdown in communication regarding actual harvest and the protocols for harvest concerning commercial licensing. However, this participant noted that the situation was quickly remedied by the leadership team.

Pilot Program Leaders

- Overall, farmer participants described their experience with the UF/IFAS program leadership team as positive. Specifically, they noted:
 - The UF/IFAS team as very receptive to their phone calls and quick to help solve any issues they had
 - If they could not be there themselves, the team would make sure they were taken care of.
 - Extension agents were very helpful in bringing energy and providing productive feedback regarding what was going on in the field compared to their personal experience.
 - Having team leaders come out to observe an issue to help resolve it was very beneficial.
 - Received a lot of help from team leadership on crop physiology

Pilot Program Recommendations

- Farmer participants recommended the development of some sort of communication network for participating farmers to share information and support one another throughout.

Industry Needs

Lastly, farmer partners were asked about their perceptions of the future and needs of the hemp industry overall. The following themes emerged from their responses:

Need for More Research

- Participants felt continued research in areas of quality control, chemical content, and plant compounds is needed to help better benefit consumers.
- A lot of participants identified plant genetics as key to moving forward with hemp in Florida, especially genetics based on region.
 - One participant expressed a need for short-day length varieties or acceptable autoflowers that would allow them to plant outside the summer window
 - This recommendation ties back to many participants’ challenges faced with planting in summer months

Reduced Restrictions

- Participants also identified reduced restrictions as one of the greatest needs for the industry, particularly the restriction on cottage food for hemp. Some participants felt this was an unnecessary restriction added by the Florida Department of Agriculture that “handcuffs farmers from coming up with creative ways to use their crops.’ as

Market Needs

- One participant in the business sector noted that a key future need in the hemp industry is finding real buyers for the product, as well as establishing win-win processing relationships
- ** Many participants expressed a desire for locally grown labeling to help move products
 - Participants emphasized the importance of the University involvement and a Fresh from Florida label for future market success.

Communication Needs

- Some participants noted a desire to develop a group or meeting of serious growers in the state to share and discuss experiences, challenges, and resources.
 - This was also identified as a way for UF/IFAS Extension to continue their involvement

Keeping Hemp U.S. Grown

- Several participants discussed their concern of hemp becoming an important crop and the ramifications of that. One participant noted, “if they start importing from Mexico, or any other country, they’re going to kill it like they killed the vegetables.”

Other Notable Findings

Research Mindset

- A lot of participants described being ready to and ok with crop loss because they approach the pilot program as a research-based, trial and error process

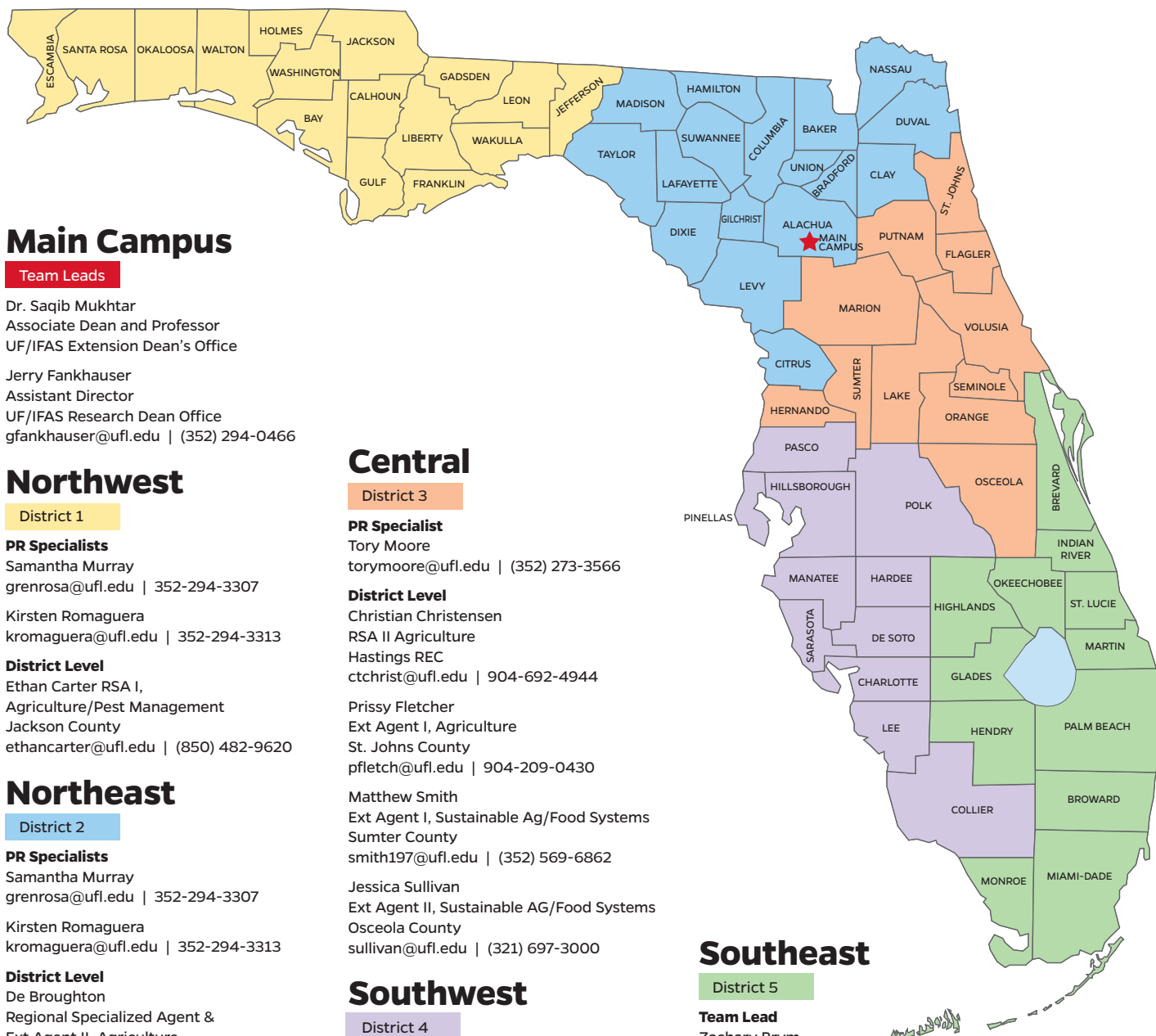
Continued Interest

- Overall, participants expressed a desire to continue working with the program into the next year and described being ready and willing to continue to try new methods for optimal plant establishment and success.

Role of Extension

- Many participants identified their Extension offers as helpful and instrumental to their success. Extension officers, both county extension and regional extension, were primarily described as being there to meet immediate needs.
 - When asked about the specific, tangible ways in which Extension supported them, participants identified the following:
 - Answering questions
 - Physically coming out to the farm to assess a situation
 - Taking and submitting samples for farmers
 - Providing information on topics (e.g., eradication of torpedo grass, links to grant funding to address an issue, etc.).
 - Helping spread information gained at other farms

UF/IFAS Statewide Hemp Communications Team



Main Campus

Team Leads

Dr. Saqib Mukhtar
Associate Dean and Professor
UF/IFAS Extension Dean's Office

Jerry Fankhauser
Assistant Director
UF/IFAS Research Dean Office
gfankhauser@ufl.edu | (352) 294-0466

Northwest

District 1

PR Specialists

Samantha Murray
grenrosa@ufl.edu | 352-294-3307

Kirsten Romaguera
kromaguera@ufl.edu | 352-294-3313

District Level

Ethan Carter RSA I,
Agriculture/Pest Management
Jackson County
ethancarter@ufl.edu | (850) 482-9620

Northeast

District 2

PR Specialists

Samantha Murray
grenrosa@ufl.edu | 352-294-3307

Kirsten Romaguera
kromaguera@ufl.edu | 352-294-3313

District Level

De Broughton
Regional Specialized Agent &
Ext Agent II, Agriculture
deonne@ufl.edu | 386-362-1725

Kevin Korus
Ext Agent II, Ag/Natural Resources
Alachua County
kkorus@ufl.edu | (352) 995-2402

D'Alicia Straughter
Ext Agent I, Family and
Consumer Sciences
Bradford County
dalicia1203@ufl.edu | 904-966-6224

Alicia N. Halbritter
Ext Agent I
Baker County Agriculture/
Natural Resources
alicia1221@ufl.edu | (904) 259-3520

Central

District 3

PR Specialist

Tory Moore
torymoore@ufl.edu | (352) 273-3566

District Level

Christian Christensen
RSA II Agriculture
Hastings REC
ctchrist@ufl.edu | 904-692-4944

Prissy Fletcher
Ext Agent I, Agriculture
St. Johns County
pfletcher@ufl.edu | 904-209-0430

Matthew Smith
Ext Agent I, Sustainable Ag/Food Systems
Sumter County
smith197@ufl.edu | (352) 569-6862

Jessica Sullivan
Ext Agent II, Sustainable AG/Food Systems
Osceola County
sullivan@ufl.edu | (321) 697-3000

Southwest

District 4

PR Specialists

Brad Buck
bradbuck@ufl.edu | (813) 757-2224

Ruth Hohl Borger
CREC-Lake Alfred
rborger@ufl.edu | (863) 956-8612

District Level

Wael Elwakil
Ext Agent II, Commercial Ag
Hillsborough County
(813) 744-5519,

Chris Prevatt
State Agent II, Budgeting and Marketing
RCREC-Ona
prevacg@ufl.edu | 863-735-1314

Southeast

District 5

Team Lead

Zachary Brym
Assistant Professor of Agroecology
TREC-Homestead
brymz@ufl.edu | (786) 217-9238

Christine Kelly-Begazo
County Ext Director III, MG Coord/Ag
Indian River County
ckellybe@ufl.edu | (772) 226-4330

PR Specialist

Lourdes Rodriguez
rodriguezl@ufl.edu | (954) 577-6363

District Level

Vanessa Campoverde
Ext Agent II, Comm AG/Ornam.
Miami-Dade County
evcampverde@ufl.edu | (305) 248-3311

Appendix G: Reports, Presentations, and Publications associated with UF/IFAS Industrial Hemp Pilot Project Efforts

Fact Sheets

UF/IFAS Industrial Hemp Pilot Project: What Farmers Should Know Before Planting. Authors: Christine Kelly-Begazo & Zack Brym. November 2019.

UF/IFAS Industrial Hemp Pilot Project: Industrial Hemp Background. Authors: Mark Tancig, Zack Brym, & Christine Kelly Begazo. December 2019.

UF/IFAS Industrial Hemp Pilot Project: Industrial Hemp Uses. Authors: Mark Tancig, Zack Brym, & Christine Kelly Begazo. December 2019.

UF/IFAS Industrial Hemp Pilot Project: Hemp Pests in Florida – Grower FAQs. Authors: Lance Osborne & Cindy McKenzie (USDA-ARS). May 2020.

Reports and Presentations

UF/IFAS North Florida Research and Education Center - 2019-2020 Industrial Hemp Report. Authors: Josh Freeman, Sarah da Silva Benevenute, & Rui Yang. May 2021.

UF/IFAS Tropical Research and Education Center – 2019 Industrial Hemp Preliminary Research Report. Authors: Zack Brym, William Wadlington, & Luis Monserrate. January 2020.

UF/IFAS North Florida Research and Education Center - 2019 Industrial Hemp Preliminary Research Report. Authors: Rui Yang, Josh Freeman, Erin Berthold, & Sarah da Silva Benevenute. January 2020.

UF/IFAS Agronomy Forage Research Unit – Phenotyping Commercial Varieties of Industrial Hemp-2019 Research Poster. Authors: Maryjo Valle, John Erickson, Zack Brym, William Wadlington, Esteban Rios, Josh Freeman. January 2020.

UF/IFAS Tropical Research and Education Center – 2020 Hemp Industry Insights Presentation. Authors: Trent Blare, Fredy Ballen, & Martha Rivera. December 2020.

UF/IFAS Bivens Arm Research Site – Industrial Hemp Pilot Project: Invasion Risk. Authors: Susan Canavan, Zack Brym, Daniel Calzadilla, Kristin Hinkson, & S. Luke Flory. December 2020.

UF/IFAS Industrial Hemp Pilot Project – Multi-Disciplinary Public-Private Sector Partnership in Industrial Hemp. Authors: Heather Kalamian, Maryjo Valle, Jordan McBreen, Keir Hamilton, Janam Acharya, Esteban Rios, Md Ali Babar, Amanda Hodges, Gerardo Celis, & Diane Rowland. December 2020.

UF/IFAS Gulf Coast Research and Education Center – Root-Knot Nematode Host Status of Different Industrial Hemp Cultivars-2020 Research Report. Authors: Jacqueline Coburn & Johan Desaegeer. December 2020.

Publications

Mylavarapu R, Brym Z, Monserrate LG, Mulvaney MJ. 2020. Hemp Fertilization: Current Knowledge,

Gaps and Efforts in Florida: A 2020 Report. Electronic Data Information Source, SL476. <https://edis.ifas.ufl.edu/ss689>

Yang R, Berthold EC, McCurdy CR, Benevenute S, Brym ZT, Freeman JH. 2020. Development of Cannabinoids in Flowers of Industrial Hemp (*Cannabis sativa* L.): A Pilot Study. *Journal of Agricultural and Food Chemistry*, 8(22): 6058–6064. <https://doi.org/10.1021/acs.jafc.0c01211>

Berthold EC, Yang R, Sharma A, Kamble SH, Kanumuri SR, King TI, Popa R, Freeman JH, Brym ZT,

Avery BA, McCurdy CR. 2020. Regulatory sampling of industrial hemp plant samples (*Cannabis sativa* L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids. *Journal of Cannabis Research*, 2, 42. <https://doi.org/10.1186/s42238-020-00050-0>

Marin MV, Coburn J, Desaegeer J, Peres, N. 2020. First Report of Cercospora Leaf Spot Caused by *Cercospora* cf. *flagellaris* on Industrial Hemp in Florida. *Plant Disease*. <https://doi.org/10.1094/PDIS-11-19-2287-PDN>

Marin MV, Wang NY, Coburn J, Desaegeer J, Peres, N. 2020. First Report of *Curvularia pseudobranchyspora* Causing Leaf Spot on Hemp (*Cannabis sativa*) in Florida. *Plant Disease*. <https://doi.org/10.1094/PDIS-03-20-0546-PDN>

Marin MV, Wang NY, Coburn J, Desaegeer J, Peres, N. 2021. First Report of *Diaporthe phaseolorum* Causing Stem Canker of Hemp (*Cannabis sativa*). *Plant Disease*. <https://doi.org/10.1094/PDIS-06-20-1174-PDN>

Azari H, Koopaei NN, Nouri MZ, Hall JD, Denslow ND, Schmittgen TD, Reynolds BA. 2020. DDRE-14. Hemp Derived Extracellular Vesicles (EVS): A Potential Anti-Glioma Therapy. *Neuro-Oncology*. <https://doi.org/10.1093/neuonc/noaa215.259>

UF/IFAS Hemp Team Faculty, Post-doctorate, and Graduate Student Articles Submitted for Publication, but Currently in Review

Anderson, S.L., B.J. Pearson, Z. Brym, and R. Kjelgren. 2021. Response of essential oil hemp (*Cannabis sativa* L.) growth, biomass, and cannabinoid profiles to varying fertigation rates. PLOS ONE. DOI: 10.1371/journal.pone.0252985.

Canavan S, Brym ZT, Brundu G, Dehnen-Schmutz K, Lieurance D, Petri T, Wadlington WH, Wilson JR, Flory SL. *In review*. Breaking the Domestication-invasion Cycle: Research Needs to Better Understand the Risk of Cannabis Cultivation. *Bioscience*.

Campbell SM, Anderson SL, Brym ZT, Pearson BJ. *In review*. Evaluation of substrate composition and exogenous hormone application on vegetative propagule rooting success of essential oil hemp (*Cannabis sativa* L.) *Plos One*.

Monserate L, Brym Z. *In review*. Agronomic performance of hemp (*Cannabis sativa* L.) varieties grown in the subtropics. *Global Change Biology Bioenergy*.

Zhang M, Anderson SL, Brym ZT, Pearson BJ. *In review*. Photoperiodic Flowering Response of Essential Oil, Grain, and Fiber Hemp (*Cannabis sativa* L.) Cultivars. *Frontiers in Plant Science*.

UF/IFAS Industrial Hemp Pilot Project: **What Farmers Should Know Before Planting**

Christine Kelly-Begazo and Dr. Zack Brym



Rules and regulations are being prepared for industrial hemp farming in Florida. Hemp may look like an exciting new crop that might be an alternative revenue stream, but it is important to understand these rules and regulations, and recognize the inherent risks associated with hemp production. Some of these risks may only be revealed well after the crop has been planted and may be costly to address after the fact. Remember that there are many undetermined factors regarding how industrial hemp will grow in Florida's unique environmental conditions under current production systems.

Make sure that you are up to date on the rules and regulations at:

<https://www.ams.usda.gov/rules-regulations/hemp>
<https://www.fdacs.gov/Cannabis/Hemp-CBD-in-Florida>

Review reference materials from the UF/IFAS Industrial Hemp Pilot Project at:

<https://programs.ifas.ufl.edu/hemp/>

Hemp Farming Remains Risky

- It is NOT legal for farmers to plant industrial hemp without a cultivation permit from FDACS.
- Seed importation and transportation will require special permits and documentation from state and federal agencies and other entities.

- It is premature at this time to recommend specific seeds or cultivars that will be successful in Florida. It is likely that varieties from northern origins will mature and flower too rapidly. Many 'varieties' are not yet stable in terms of plant growth or THC development. The goal is a certified variety from sub-tropical environments that consistently performs well in Florida with THC that does not exceed 0.3% total THC by dry weight.
- Hemp plantings grown in a defined area that surpass the legal limit of 0.3% total THC by dry weight will have to be disposed of, perhaps under the supervision of someone authorized to handle controlled substances. Hemp plants can surpass the legal limit (i.e., test over 0.3% total THC by dry weight) due to genetics or a variety of stress driven by factors such as heat, moisture, nutrition, or pests. Testing for THC levels will be performed per USDA and FDACS requirements.
- Due to the lack of knowledge on how industrial hemp will perform in Florida, the potential for it to escape from cultivation and become invasive must be seriously considered. In other states and countries, hemp has been, or is, regarded as a weed. Other possible environmental impacts of its introduction in Florida should be considered. Careful consideration and effective control measures must be embraced in order to avoid future problems in nearby cultivated and natural areas.

- Hemp plantings may require additional security measures to deter trespassing and theft.
- Registered agrochemicals or pesticides labeled for use on industrial hemp are extremely limited. Weeds, pests and diseases will need to be controlled by other mechanical, physical, cultural and biological methods.
- Financial institutions willing to loan money to industrial hemp farmers are limited due to the risk of suspicious activity. Federal crop insurance may be available to farmers on a multi-crop insurance program.

Be Prepared

- Learn all you can about industrial hemp. The UF/IFAS Hemp Pilot Project is here to help but there is still a lot to research and understand. To stay up to date with the latest research from UF/IFAS, subscribe at <https://programs.ifas.ufl.edu/hemp/newsletter>.
- Each grower will have to apply for a permit from FDACS in order to grow industrial hemp and comply with the state and federal regulations. Refer to their website for the application procedure and process.
- Growing flowers to extract CBD, and/or other components, is very different than growing for fiber and grain. The specific cropping system will depend upon the type of hemp a producer wants to grow. It is best to select what type of hemp you want to grow based upon sound market availability and then learn about the production methodology associated with it.
- Get your soil fertility and water source tested before planting. The UF/IFAS Extension Soil Testing Laboratory offers this service <http://soilslab.ifas.ufl.edu/ESTL%20Tests.asp>, click on “Producer Soil Test”.
- Due to Florida’s predominately sandy soils, nematodes (microscopic plant-parasitic roundworms) might be a problem and it will be important to have your soil checked for those as well via a nematode assay. For more information visit the UF/IFAS Entomology & Nematology Nematode Assay Lab at http://nematology.ifas.ufl.edu/assaylab/Sample_Submission.html.
- Purchase only certified or pilot project approved seeds and plants from reputable sources that meet FDACS rules.
- Start with a small amount of acreage and learn as you go for your specific operation.

- Make sure you have a market or buyer before you make investments to cultivate hemp.
- Have all contracts reviewed by an experienced legal authority.
- Local law enforcement agencies will need to be educated on the differences between hemp and marijuana and a list of permitted farmers will need to be available to them so they can cross-check against suspected illegal operations.
- There are many claims as to the success of growing hemp and its economic returns. Before jumping into any venture, is it important to look at all aspects and angles to make sure that it will be profitable for your current situation.
- Talk to other farmers in states where it is currently legal to grow hemp and hear what they have to say about this crop.
- Consult with your local and regional UF/IFAS Extension county agents and faculty.



UF/IFAS Industrial Hemp

Industrial Hemp Background

by Mark Tancig, Zack Brym and Christine Kelly-Begazo



Taxonomy of *Cannabis sativa* (L.)

The hemp plant is botanically described as *Cannabis sativa*. The taxonomic classification of *Cannabis* has been debated among botanists due to possible differences in origin and morphology. However, while there are many strains with unique characteristics, they all are capable of inter-breeding, and therefore do not meet the typical biological definition of species. The most current classification considers all strains, including hemp and marijuana, as one species, *Cannabis sativa*, as originally named by Carl Linnaeus.

In many of the states and countries where hemp is permitted to be grown, hemp is defined by statute as those *C. sativa* plants that have THC levels below 0.3%, on a dry-weight basis. Hemp has been historically grown for fiber and seed production. It has low levels of the psychoactive component delta-9-tetrahydrocannabinol (THC) and high levels of cannabidiol (CBD), a non-psychoactive compound that has potential therapeutic uses.

History

For centuries, humans have cultivated *C. sativa* for fiber, food, seed oil, medicine, and ritual. Archaeologists and historians have confirmed the use of hemp for fiber and food by ancient civilizations as early as 8000 BCE, with mentions in ancient texts of advanced industrial applications as early as 500 BCE. Fiber from hemp was critical in the domestication of animals and establishment of naval transportation. In 1545, hemp arrived in the Americas via the Spanish. Hemp was noted as an important fiber crop, along with flax, in the New England colonies by the mid-1600s. The first two copies of the Declaration of Independence were printed on paper made of hemp.

Peak hemp production in the United States was in the mid-1800s with temporary spikes during both World Wars. Industrial hemp production was most common in Illinois, Iowa, Indiana, Minnesota, Wisconsin, and Kentucky—which had the highest production. The cultivation of hemp, primarily for fiber, was common

worldwide up until the 1820s and the introduction of other fiber crops (jute, sisal, cotton) and synthetic fibers led to a decline in hemp demand and production. It has been documented that 75,000 tons of hemp was produced in the 1840s while only 2,000 tons were produced in 1948.

Regulation

In addition to market forces, statutes and regulations enacted in the early 20th century such as the Marijuana Tax Act and the Controlled Substances Act to control access to *C. sativa* led to the ultimate demise of the United States hemp industry. Regulations, coupled with the taxonomic ambiguity described previously, created legal challenges for cultivating or possessing any *C. sativa*.

Changes in public opinion regarding *C. sativa* regulation and potential medicinal use led some states to pass legislation allowing the cultivation and sale of medicinal *C. sativa* in the late 1990s. This has led to proponents of hemp to advocate for the ability to grow non-psychoactive *C. sativa* for other uses and as a potentially important and profitable alternative agriculture product. The 2014 and 2018 Farm Bills included language to distinguish hemp from marijuana and to provide a framework for the legal cultivation of hemp (<https://www.ams.usda.gov/rules-regulations/hemp>).

In 2017, the Florida legislature approved SB 1726 – *Industrial Hemp Pilot Projects*, allowing land grant universities to conduct industrial hemp pilot projects. UF/IFAS has developed a strong research program in support of the future industry and collecting information for future farmers. Producers considering industrial hemp as a possible commodity, can keep up with the latest information available from FDACS at <https://www.fdacs.gov/Cannabis/Hemp-CBD-in-Florida> and the UF/IFAS Industrial Hemp research team at <https://programs.ifas.ufl.edu/hemp/>.



Large-scale industrial hemp propagation for CBD oil in North Carolina. Photo credit: Christine Kelly-Begazo

References

- Ash, A. L. (1948). Hemp-production and utilization. *Economic Botany*, 2(2), 158–169. <https://doi.org/10.1007/BF02858999>
- De Candolle, A. (1886). *Origin of Cultivated Plants*. Hafner Publishing Company. <https://doi.org/10.1017/CBO9781107415324.004>
- Ehrensing, D. T. (1998). *Feasibility of Industrial Hemp Production in the United States Pacific Northwest*. Retrieved from <https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/sb681.pdf>
- Johnson, R. (2014). *Hemp as an Agricultural Commodity*. Washington, DC. Retrieved from <https://apps.dtic.mil/docs/citations/ADA599368>
- Kraenzel, D. G., Petry, T., Nelson, B., Anderson, M. J., Mathern, D., & Todd, R. (1998). *Industrial Hemp as an Alternative Crop in North Dakota: A White Paper Study of the Markets, Profitability, Processing, Agronomics and History*.
- Li, H. L. (1973). An archaeological and historical account of cannabis in China. *Economic Botany*, 28(4), 437–448. <https://doi.org/10.1007/BF02862859>
- McPartland, J. M., & Guy, G. W. (2017). Models of Cannabis Taxonomy, Cultural Bias, and Conflicts between Scientific and Vernacular Names. *Botanical Review*, 83(4), 327–381. <https://doi.org/10.1007/s12229-017-9187-0>
- U.S. Department of Agriculture. (1909). *Seeds and Plants Imported During the Period from October 1 to December 31, 1908: Inventory No. 17; Nos. 23745 to 24429*. Washington, DC. Retrieved from <https://files.eric.ed.gov/fulltext/ED541499.pdf>
- U.S. Department of Agriculture. (1914). *Yearbook of the United States Department of Agriculture - 1913*. Washington, DC.

UF/IFAS Industrial Hemp

Industrial Hemp Uses

by Mark Tancig, Zack Brym and Christine Kelly-Begazo



Cannabis sativa has been collected and grown since the earliest days of agriculture. Hemp has been recently distinguished as *Cannabis sativa* with THC <0.3%, which relates to primary uses for fiber and grain. Additional uses in modern cultivation include, biocomposite plastics, seed oil, biofuel production, and essential oil containing cannabinoids such as cannabidiol (CBD). The following sections describe the current uses of hemp characterized by their source – stem, seed, or inflorescence.

Stem

Hemp fibers arise from the stems. The two types of fibers that can be used for processing are the hurd (inner pith of short, woody fibers) and the bast (outer phloem) fibers. Hurd fibers are used for fiberboard, compost, paper filler, absorbent, animal bedding, and as a chemical component of plastics, paints, and sealants. These higher quality bast fibers are from the inner bark and have been described as strong, lustrous and very durable. Bast is considered a softer fiber (from stems), like jute (*Corchorus* spp.) and flax (*Linum usitatissimum*), rather than a hard fiber (from leaves), such as sisal (*Agave sisalina*) or abaca (*Musa textiis*). Bast fibers can

be used for specialty paper, fabric, insulation, carpeting, cordage, and pulp.

Industrial hemp biomass, the combined harvest of stems and leaves, is also useful as a biofuel crop and has comparable yields as other lignocellulosic, non-food crops used to produce biofuels like sorghum and switchgrass.

Seed

Hemp seeds are utilized as a grain or for their high-quality oil, and should not be confused with CBD oil. The use of hempseed for grain dates back thousands of years, and is still used in traditional Asian foods today. Hempseed is approximately 30% oil, 25% protein, and contains dietary fiber, vitamins, and minerals. The oil pressed from the grain (botanically an achene) is high in polyunsaturated fatty acids and contains two essential fatty acids, linoleic (an omega 6) and α -linolenic (an omega 3) acid.

The high concentration of polyunsaturated acids contained in the oil can be used for various industrial applications, including varnishes and paint drying agents.

Inflorescence



Photo Credit: Christine Kelly-Begazo

The inflorescence of the hemp plant, comprised of the flower stalk, along with leaves, can be processed via several extraction methods (CO₂, ethanol, etc.) to produce various cannabinoids, including CBD and THC. CBD lacks the psychoactive properties of THC and has been shown to provide therapeutic and medicinal benefits.

References

- Amar, M. Ben. (2006). Cannabinoids in medicine: A review of their therapeutic potential. *Journal of Ethnopharmacology*, 105(1–2), 1–25. <https://doi.org/10.1016/j.jep.2006.02.001>
- Callaway, J. C. (2004). Hempseed as a nutritional resource: An overview. *Euphytica*, 140, 65–72. Retrieved from <http://eds.a.ebscohost.com/eds/detail/detail?vid=8&sid=efaf575d-15f4-4ab2-9fee-a45fdf793e22%40sessionmgr4002&hid=4110&bdata=Jmxhbm9Y3Mmc2l0ZT1lZHMtbGl2ZQ%3D%3D#AN=16342637&db=a9h>
- Carus, M., Karst, S., Hobson, J., & Bertucelli, S. (2013). *The European Hemp Industry: Cultivation, processing and applications for fibres, shivs and seeds*. European Industrial Hemp Association. <https://doi.org/10.1016/j.jallcom.2015.04.081>
- Das, L., Liu, E., Saeed, A., Williams, D. W., Hu, H., Li, C., ... Shi, J. (2017). Industrial hemp as a potential bioenergy crop in comparison with kenaf, switchgrass and biomass sorghum. *Bioresour Technol*, 244, 641–649. <https://doi.org/10.1016/j.BJM-12-2017-0397>
- Fortenbery, T. R., & Bennett, M. (2004). Opportunities for commercial hemp production. *Review of Agricultural Economics*. <https://doi.org/10.1111/j.1467-9353.2003.00164.x>
- Leizer, C., Ribnicky, D., Poulev, A., Dushenkov, S., & Raskin, I. (2000). The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition. *Journal of Nutraceuticals, Functional & Medical Foods*, 2(4), 35–53. https://doi.org/10.1300/J133v02n04_04
- Pisanti, S., Malfitano, A. M., Ciaglia, E., Lamberti, A., Ranieri, R., Cuomo, G., ... Bifulco, M. (2017, July 1). Cannabidiol: State of the art and new challenges for therapeutic applications. *Pharmacology and Therapeutics*. Pergamon. <https://doi.org/10.1016/j.pharmthera.2017.02.041>
- 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*. <https://doi.org/10.1016/j.rser.2012.10.019>

UF/IFAS Industrial Hemp Pilot Project: Hemp pests in Florida – Grower FAQs

Lance Osborne, UF/IFAS Mid-Florida Research & Education Center, Isosborn@ufl.edu and Cindy McKenzie, Ph.D., USDA-ARS, cindy.mckenzie@usda.gov

Florida growers with hemp permits will begin to obtain plant material as soon as possible. Unfortunately, pest management is often not considered until it is too late.

Over the last year, the **UF/IFAS Industrial Hemp Pilot Project** team learned the hard way how important it is to have a pest management plan prior to importing hemp into an operation.

The day after receiving the first stock plants at the UF/IFAS Mid-Florida Research and Education Center in Apopka, researchers found both aphids and whiteflies. The plants had already passed phytosanitary inspections by regulatory staff in the states of origin and in Florida.

This fact sheet specifically applies to plant material. There are a few pest issues that will impact seeds, but UF/IFAS has observed significantly more issues from arthropods on plants and cuttings both rooted and unrooted.



Left to right, top to bottom: Hemp russet mite, Glover mite on hemp plants, Glover mite, Omnivorous leaf roller and Rice root aphid

What pests may I find on hemp plants?

Unfortunately, most of the hemp plant shipments received by UF/IFAS have been infested with either the cannabis aphid or the hemp russet mite. Both of these species are not established in Florida and are thus considered actionable pests. Actionable pests being present means that the plant material will be quarantined and not allowed to be moved until certified to do so.

What should I do before ordering hemp plants?

A pest management strategy must be in place prior to purchasing plants or cuttings. First, talk to your supplier to develop a plan to minimize the risk of importing unwanted pests. There are consequences for suppliers who ship infested hemp plants to Florida, so it is in their best interest to work with you.

The pesticides that the hemp industry can use are limited and require many applications to reach nondetectable pest levels. There is no guarantee that the supplier will eradicate every pest and ship completely pest-free plant material.

All pests should be identified by your supplier and by you if detected in your crop. Only heavily infested plant materials or plants infested with pests not found in Florida will result in regulatory action by FDACS. You can set guidelines as to what you will accept as long as it is within the FDACS parameters. Suppliers can share with you what methods seem to work in the control of various potential pests. Any materials used have to be legal for use on hemp before you can use them in Florida according to FDACS guidelines.

How do I handle hemp plants once they arrive?

Within your facility, set up a location to quarantine all new plant material as it arrives. This is something UF/IFAS recommends to ornamental and greenhouse vegetable growers as well. This gives you the opportunity to inspect new plants for a few weeks, and increases your probability of identifying any problems, thus reducing the risk of contaminating your growing facility.



Growers and scouts should use a high quality 10x or 20x hand lens when scouting for pests.

Should I scout for pests after receiving my hemp plants? How often and why?

Scouting for arthropod pests is the foundation for managing them both efficiently and economically. Growers and scouts should use a high quality 10x or 20x hand lens. Our favorite lens generally costs more than \$20, but their quality is well worth the extra money. We purchase ours from scientific supply houses such as Fisher Scientific, Forestry Suppliers and Bioquip Products. As these are very tiny pests, we recommend two hand lenses: a 10x and 20x.

Why continue to scout?

Besides the obvious need to detect problems before they are so bad you are at risk of losing your crop, you need to be able to determine if your control tactics are working. Knowing what pests are present will dictate what control measures will help mitigate the damage they cause.

What happens if I find pests on my hemp plants?

Immediately quarantine the plants that have pests if they are not already quarantined.

Upon finding pests on the UF/IFAS hemp stock plants, the UF/IFAS greenhouses were quarantined. Nothing could be moved out of the greenhouses until the state inspectors found no pests after at least three consecutive inspections. It took months before the plant material was clean from aphids or mites and able to be moved out of the greenhouses.

What can I do to reduce pest risk?

In greenhouses

It is much easier to prevent pest problems than it is to cure them. UF/IFAS does not allow visitors to the stock plant growing facility. We have no idea where people have been prior to coming to visit and they could bring in a pest with them.

A method to consider that is also used in citrus greenhouses, upon entry, you are sprayed with a disinfectant and you must step in a disinfectant-containing foot bath prior to entry. This is mainly to prevent tracking in diseases but it's also another example of how you should try to prevent pests from gaining entry if you can.

When handling or transporting hemp plants

In some cases to help kill unwanted pests, we dip cuttings in soaps or oils prior to rooting or transplanting them into larger containers. This is also a common practice in the Florida ornamental industry with the directions for doing so included on some pesticide labels. We have published several papers evaluating this method to kill such pests as mealybugs, **whiteflies** and mites. In general, we found that soaps and oils were both safe and effective. Certain pesticides might work better, but the safety to both workers and the plant material is an issue that has limited our interest in evaluating such treatments. This tactic is just an additional tool in a systematic approach to managing the risk of importing unwanted pests on new plant material.

What else can I do to be prepared for hemp pests?

We recommend making a list of the pests you might potentially find attacking hemp in Florida. Next, make a list for each pest of the pesticides that are legal, approved in Florida and available to you. Pesticide labels will list which pests it has activity on, the rates to be used and other use directions, precautions and restrictions. This will reduce the number of materials on your list. UF/IFAS and FDACS publications and consultants can provide some help with developing a list of materials registered in Florida.

Once you have a list, check the supply chain for each material. Some of the pesticides listed may only come in small quantities or they may be very difficult to find in Florida. Once you have a small quantity of the pesticide, treat a small number of plants with the material using the frequency and all other labeled instructions to determine plant safety (phytotoxicity) in your particular environment.

Additional resources

The Florida pesticide list is not all inclusive. There are materials that may be legal and potentially useful in Florida hemp production, but FDACS is adding materials to the list as they make decisions on their legal usage. FDACS publishes a list of products they have approved for use on hemp (**Pesticide Products for Use on Hemp**). They have also developed a document that explains the criteria they use to make their decision about adding a product to their list (**Pesticide Brochure**).

Industrial Hemp Research Report

Josh Freeman

Associate Professor of
Horticultural Sciences,
University of Florida,
North Florida Research
and Education Center

Sarah da Silva Benevenuto

Graduate Research
Assistant, University of
Florida, North Florida
Research and Education
Center

Rui Yang

Institute of Urban
Agriculture, Chinese
Academy of Agricultural
Sciences, Chengdu,
Sichuan 610200, China

Introduction

Industrial hemp is legally classified in the USA as *Cannabis spp.* with tetrahydrocannabinol (THC) concentration of $\leq 0.3\%$ per dry weight basis, cannabis plants that exceed the 0.3% THC threshold will no longer be classified as industrial hemp (IH). IH is a potential new crop for the state of Florida, and to support the future viability of this crop appropriate agronomic practices including plant density, varieties, and pruning (pinching) practices need to be determined to achieve successful production. Most varieties of IH are sensitive to day length, meaning they remain vegetative during the long days of summer, and they flower when days begin to shorten. In north Florida, for the varieties tested so far, growing season is defined to be around late May to around the first or second week of August. During this time days are long enough to maintain plants in a vegetative stage, before and after that, plants will flower.

Open field IH production exclusively for essential oils is a new venture in the United States for growers and researchers, and much of what is practiced by growers is deduced from indoor marijuana production systems. It is known that greatest cannabinoid content is usually found in unpollinated female flowers and is often found at much lower concentration in other tissues of female or male plants. Pollination is detrimental to essential oil production therefore male plants should be avoided in the field in order to reduce chances of pollination.

There is a significant economic risk in hemp production due to the high cost of production and the potential for the crop to exceed the 0.3% THC threshold, and therefore be unmarketable.

Experiments were conducted during the 2019 and 2020 growing season at the North Florida Research and Education Center (NFREC) in Quincy, Florida on IH varieties for essential oil production.

It should be noted that the research presented in this document was obtained from only two seasons, and from a limited number of IH varieties. There will certainly be variation between seasons, locations, and IH varieties. The mention of variety names in this document is not meant to serve as an endorsement nor are these data to be considered a recommendation.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Objectives

Evaluate IH varieties, the impact of pinching, and plant density on flower yield and cannabinoid content of IH cultivated under open field conditions in northern Florida.

Materials and Methods

For all field experiments, feminized seeds were germinated and grown in a greenhouse with supplemental lighting using peat based potting media. Seedlings were produced in 128 cell trays with only half of the tray planted to increase space for each seedling. Uniform seedlings were transplanted to the field at around 21 days after seeding on July 3, 2019 and July 14, 2020. All experiments were produced utilizing raised beds and the plasticulture production system that is typical for many vegetable crops. Raised beds were 8 in tall and 30 in wide and were covered with white colored plastic to reduce the soil temperature. Fertilizer (N-P₂O₅-K₂O: 10-10-10) was applied under the plastic prior to planting and soluble fertilizer was delivered through the irrigation system during the growing season. Total fertilizer application for the season was 150, 100, and 200 lb/acre for N, P₂O₅, and K₂O, respectively for each growing season. Irrigation was provided to the crop, up to 1.25 acre inches per week, through a single drip tubing located under the plastic.

Variety Study

As with any crop variety selection is critical to achieve successful production. However, there is no history of IH in this area, so growers don't have the necessary information to make decisions on variety selection, planting date, plant density, or other crop management practices. In 2020, eight day-length sensitive IH varieties (Cherry Blossom, Cherry Wine, Berry Blossom, Hot Blonde, Cinderella Story, Cloud Berry, Queen Dream, Cherry Blonde) were evaluated. Seedlings were transplanted to the field on July 14th and plants were harvested on October 1st, 2020. For this study plants were spaced 3 ft within the row and rows were spaced 6 ft apart (~2420 plants per acre). Flowering began around August 13th, and all varieties initiated flowering within one week of each other. Plants were harvested based on pre-harvest THC sampling, dried in a forced-air drier at 130 °F for 72 h, flowers were removed by hand and flower yield was recorded. Flowers and leaves were then ground into fine powder for cannabinoid analysis.

USDA allows a measurement of "uncertainty" (analytical error) in addition to the result. The analytical method used in our study has an uncertainty of 0.05%, therefore, the THC threshold of 0.35% was used in the following comparison.

All tested varieties in this trial tested above THC threshold upon harvest. No differences in THC, CBD and other cannabinoids were observed among the tested varieties. Weekly testing was performed to evaluate the THC concentration and THC threshold was exceed around 3-5 weeks post flowering initiation, which was similar to the 2019 season. For most of the varieties tested, when THC concentration goes above the threshold it doesn't go back below this limit. Growers should carefully monitor THC concentrations to avoid having crops exceed THC threshold.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Flower yield and cannabinoid concentration of industrial hemp grown in Quincy, FL during 2020.

Variety	Flower yield	Total THC	Total CBD	Total CBC	Total CBG
	lb/acre	-----% dry weight----- ---			
Cherry Blonde	1932 a	0.56	12.6	0.56	0.30
Cinderella Story	1761 ab	0.55	12.2	0.60	0.19
Cloud Berry	1712 ab	0.50	11.3	0.57	0.23
Cherry Blossom	1693 ab	0.43 ^{ns}	10.1 ^{ns}	0.54 ^{ns}	0.22 ^{ns}
Queen Dream	1560 ab	0.40	9.4	0.50	0.25
Hot Blonde	1483 bc	0.44	10.1	0.49	0.20
Cherry Wine	1371 bc	0.45	10.3	0.55	0.21
Berry Blossom	1158 c	0.41	9.3	0.43	0.19

ns = no statistically significant differences detected, means are to be compared within columns.

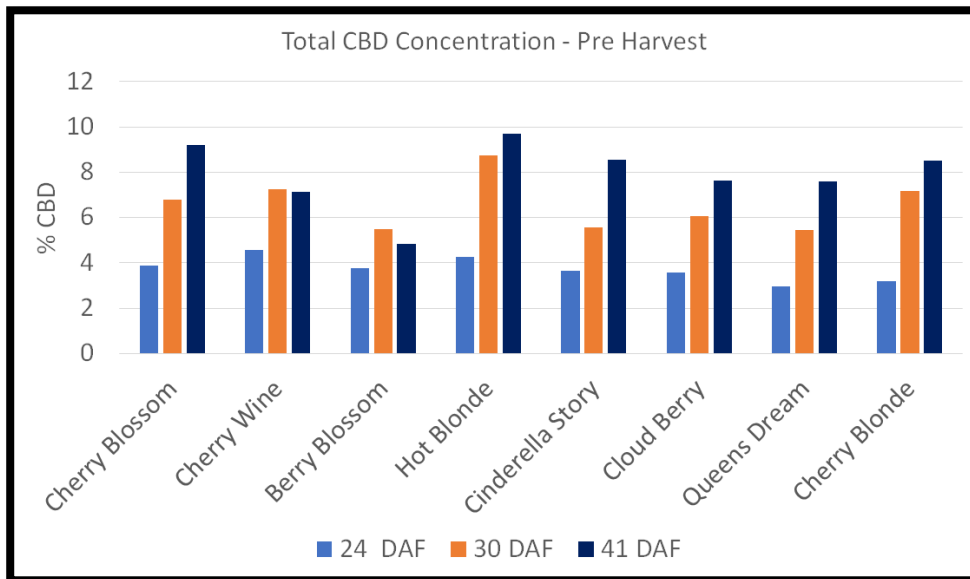
For more information visit: <https://programs.ifas.ufl.edu/hemp/>

THC and CBD Development

Prior to harvest, flower samples were taken from the top 8" of plants from each variety on a weekly basis and tested for cannabinoid concentration. Our goal with these samples was to time our harvest where THC concentration was below the established threshold. Results showed that both total CBD and total THC content increased with time, as flowers mature.

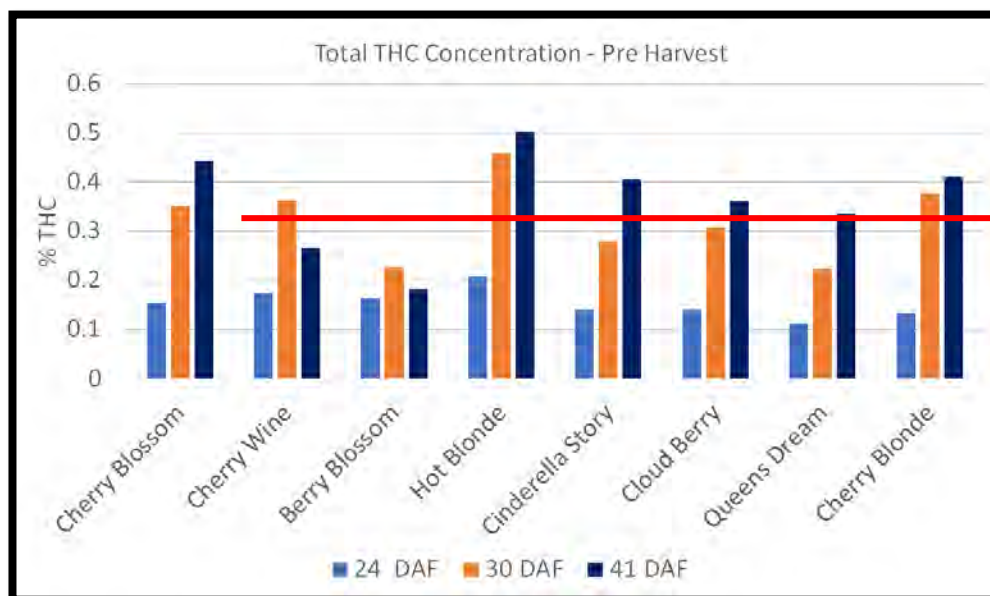
We noticed a significant increase in total THC between 24 and 30 days after flowering initiation, in which total THC content in Cherry Blossom, Cherry Wine, Hot Blonde, and Cherry Blonde went above the legal threshold. The total THC content in other tested varieties went above the threshold in the following week, between 30 and 40 days after flowering. Once THC content in industrial hemp plants goes above threshold it rarely goes back below threshold in the following weeks. Only in two varieties (Cherry Wine and Berry Blossom) did the THC content decrease from one week to next.

This data illustrates the need to sample industrial hemp frequently prior to harvest to maintain a legal, marketable crop.



DAF = days after flowering.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>



DAF = days after flowering.

Pinching Study

A common practice among cannabis producers is to remove (known as pinching or topping) the apical meristem in the main shoot of the plant in early stages of plant development to improve flower formation on lateral branches and increase flower yield per plant. However, in high CBD type industrial hemp varieties grown in open field conditions, its unknown if this practice would have any effect on yield or cannabinoid content.

Two day-length sensitive varieties, Cherry Blossom (CBL) and Cherry Wine (CW) were evaluated due to their popularity among growers, and availability of feminized seeds. Pinching was performed 21-27 days after transplanting (DAT), with the apical meristem plus 2-3 subsequent internodes removed with pruning shears. Plants were spaced 5 ft apart within row and rows 6 ft apart, resulting in 1,450 plants per acre.

Plants were harvested 8 weeks after anthesis, dried in a forced-air drier at 130 °F for 72 h, flowers were removed by hand and flower yield was recorded. Flowers and leaves were then ground into fine powder for cannabinoid analysis.

In 2109, plant height and flower yield were significantly greater than those in 2020, which could be due to a later planting date in 2020. Previous research has demonstrated that early planting dates may result in greater flower yield compared to late planting dates in northern Florida. In 2019, CBL had greater plant height, and flower yield compared to CW, but no difference was observed in THC and CBD content among the two varieties. However, in 2020 while plant height, and flower yield did not significantly differ between the two varieties, total THC and CBD was significantly greater in CBL compared to CW.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

The effect of industrial hemp variety on plant height, flower yield, and cannabinoid concentration from experiments conducted in Quincy, FL during 2019 and 2020.

Year and variety	Plant height	Flower yield	Total THC	Total CBD	Total CBC	Total CBG
	inches	(lb/plant)	-----% dry weight-----			
2019						
CBL	53.5 a	1.96 a	0.561 a	11.454 a	0.712 ^{ns}	0.242 ^{ns}
CW	47.2 b	1.52 b	0.510 a	10.950 a	0.774	0.230
2020						
CBL	35.8 c	0.85 c	0.541 a	12.614 a	0.780	0.274
CW	36.6 c	0.88 c	0.316 b	7.953 b	0.658	0.189

ns = no statistically significant differences detected.

There was no interaction between IH variety, pinching, and year, so the data were combined to illustrate the main impact of pinching. Pinching showed no significant effect on yield traits or total cannabinoid concentration except that pinched plants were shorter relative to non-pinched plants. Since pinching can increase labor expenses, a lack of yield improvement could lower the overall economic return.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

The effect of pinching on yield and cannabinoid concentration of industrial hemp grown in Quincy, FL during 2019 and 2020.

Treatment	Plant height	Flower yield	Total THC	Total CBD	Total CBC	Total CBG
	inches	(lb/plant)	-----% dry weight-----			
Non-pinched	46.0 a	1.32 ^{ns}	0.527 ^{ns}	11.225 ^{ns}	0.789 ^{ns}	0.257 ^{ns}
Pinched	41.3 b	1.33	0.488	10.703	0.696	0.217

ns = no statistically significant differences detected.

Plant Density Study

Appropriate plant density is a critical factor that can affect crop yield, and consequently, profitability. Currently, in the southeast USA, industrial hemp fields for essential oil production are established between 1,000 and 2,000 plants per acre but it is still unknown if that is the most appropriate plant density. For the plant density study, only Cherry Wine (CW) was evaluated. Four plant densities, including 1,210, 1,613, 2,420, and 4,840 plants per acre were achieved by using different in-row spacing (1.5, 3.0, 4.5, and 6.0 ft). Rows were spaced 6 ft apart. Seedlings were transplanted to the field at around 21 days after seeding on July 3, 2019 and July 14, 2020.

Flower yield per acre gradually increased with increasing plant density (more plants per acre led to greater total flower yield). The greatest plant density (4,840 plants per acre) resulted in the greatest flower yield per acre, and the two lower plant densities (1,210 and 1,613 plants per acre) did not significantly differ from each other in total flower yield. This trend, however, reversed on a per-plant basis. The greatest plant density tested in this study (4,840 plants per acre) produced the lowest flower yield per-plant (less plants per acre led to more flower per plant). Flower yield per plant were not significantly different among the three lower plant densities tested (1,210, 1,613, and 2,420 plants per acre). Plant density did not have a significant impact on total cannabinoid concentration. Plant height also tended to increase with increasing plant density, but this trend did not reach statistical significance.

It is likely that planting date will interact with plant spacing because hemp is day-length sensitive. If the same trial was conducted with a late May planting date, a lower plant density may be more appropriate. Future research is needed to determine appropriate IH plant density for different planting dates.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

The effect of plant density on yield and cannabinoid concentration of industrial hemp grown in Quincy, FL during 2019 and 2020.

Plant density	Plant height	Flower yield	Flower yield	Total THC	Total CBD	Total CBC	Total CBG
plants / acre	inches	lb/plant	lb/acre	-----% dry weight----- ---			
1,210	39.7 ^{ns}	0.9 a	1,267 c	0.489 ^{ns}	10.798 ^{ns}	0.729 ^{ns}	0.193 ^{ns}
1,613	40.1	0.9 a	1,497 c	0.464	10.175	0.710	0.199
2,420	41.3	0.89 a	2,108 b	0.442	10.188	0.631	0.194
4,840	42.9	0.6 b	3,482 a	0.472	10.236	0.687	0.220

ns = no statistically significant differences detected.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Preliminary Research Report

Zachary Brym

Assistant Professor
of Agronomy,
University of
Florida, Tropical
Research and
Education Center

William**Wadlington**

Post-doctoral
Associate,
University of
Florida, Tropical
Research and
Education Center

Luis Monserrate

Graduate Student,
University of
Florida, Tropical
Research and
Education Center

Industrial hemp trials were initiated in spring 2019 at the University of Florida Institute of Food and Agricultural Sciences Tropical Research and Education Center (TREC) located in Homestead, Florida (25.4687° N, 80.5007° W). A direct seeded variety trial and planting date trial were carried out simultaneously throughout the season. Measurements were taken to assess plant performance, including flowering date, cannabinoid concentration, stand establishment, and yield.

Varietal differences were found in terms of plant development, growth, and yield. Preliminary results do not point to a clearly superior variety that is well adapted for South Florida, is sufficiently productive, and meets legal requirements for industrial hemp. Several varieties showed promise for future trials and breeding efforts. Differences among varieties were largely driven by flowering behavior. The total THC concentration of some, but not all, varieties exceeded 0.3%, the current legal definition for industrial hemp. Top performing varieties generally did better when planted in May before the hot rainy season. Several varieties had competitive or exceptional yield compared to commercial targets for fiber or grain harvest.

It should be noted that the research presented in this document was obtained from a single field season, at a single location, and from a limited number of hemp varieties. There will certainly be variation between seasons, locations, and hemp varieties. **The mention of variety names in this document is not meant to serve as an endorsement nor are these data to be considered a recommendation. Additionally, hemp is predicted to have a high risk of invasion in natural areas according to the UF/IFAS Assessment of Non-Native Plants in Florida's Natural Areas and may be problematic as an agricultural weed. Thus UF/IFAS will continue to conduct research on invasive risk, variety selection, and management options for FL conditions to address these concerns.**

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Variety trial

Methods

The variety trial was planted at TREC on May 22nd, 2019 with 23 hemp varieties representing diverse latitude of origin from regions across the world including North America, Europe, and Asia and the various purposes of fiber, grain, and essential oil production (Table 1). The hemp varieties in the trial were not specifically selected for adaptive qualities for Florida, but rather the varieties accessible to the research program for the 2019 planting season. Plants in the variety trial were monitored for flowering behavior and, when ample material was available, sampled for cannabinoid concentration.

The environment at UF/IFAS TREC is characterized by a subtropical climate with a wet season from May to October, a mean annual temperature of 74.1°F, and a rainfall of 65 in. Soil is rocky, called rockdale or Krome gravelly loam, and is derived from Miami oolitic limestone. Soil depth is 6-12 in resulting from excavation and rock plowing. The land was cultivated, disked, and fertilized prior to planting. Fertilizer was broadcast at a rate of 112 lb N, 56 lb P₂O₅, and 300 lb K₂O per acre with a slow release granular fertilizer. Plots were irrigated with overhead sprinklers as needed to keep soil moist. Each variety was planted in four 6 ft x 10 ft plots. Planting density was 1500, 900, and 60 seeds per plot for fiber, grain/dual, and essential oil, respectively. The seeding rate of 1500 seeds per plot is approximately equivalent to 60 lbs/ac, while 900 seeds per plot is approximately 35 lbs/ac. Fiber and grain seeds were evenly distributed along 8 rows separated by 8 inches. CBD seeds were planted into mounds at 2 ft x 2 ft spacing.

Flowering behavior

Plots were monitored frequently for flower development and recorded as percent of each plot at floral induction determined visually. Floral induction was determined for individual plants when the upper nodes changed to indicate the initiation of flower development. Flowering date was defined when the plot reached 50% of individuals with flower induction (Table 1).

Hemp varieties in our trial demonstrated flowering behavior indicative of short-day photoperiod sensitive plants. Short-day hemp plants require the long days of summer to grow vegetatively before flowering as days shorten to a critical length. Differences in flowering date were largely driven by latitude of origin (Table 1, Fig 1). Because the critical day length for flowering in varieties adapted to northern latitudes is high, we expected them to flower early given the lack of those long days in Florida. No Canadian or northern European varieties grew later than a few weeks before flowering which is far short of harvestable size or maturity. In contrast, some southern European and Chinese varieties showed a month or more of growth prior to flowering. Latitude of origin was an indicator of the date each variety flowered. Time to flowering impacted production outcomes (i.e., fiber vs. grain) and yield.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Table 1. List of varieties planted and evaluated in the variety trial with their origin, production purpose and date of flowering. Date of flowering was determined by the earliest date that 50% of the plants in a plot demonstrated signs of floral induction.

Variety	Origin	Purpose	Trial Flowering
CFX-1	Canada	Grain	6/10/19
CFX-2	Canada	Grain	6/11/19
Joey	Canada	Dual	6/11/19
Canda	Canada	Dual	6/12/19
CRS-1	Canada	Grain	6/12/19
Carmagnola	Italy	Dual	6/13/19
Han FN-Q	North China	Dual	6/13/19
Carmagnola Selezionata	Italy	Dual	6/14/19
Helena	Serbia	Dual	6/14/19
Tygra	Poland	Dual	6/14/19
Han FN-H	North China	Dual	6/15/19
Fibranova	Italy	Fiber	6/17/19
Eletta Campana	Italy	Fiber	6/19/19
Han NE	Central China	Dual	6/24/19
Han NW	Central China	Dual	7/03/19
Cherry Blossom x T1	USA	Essential oil	7/27/19
Berry Blossom	USA	Essential oil	7/31/19
Puma-3	South China	Fiber	8/29/19
Puma-4	South China	Fiber	8/31/19
Bama	South China	Dual	9/07/19
Yuma	South China	Dual	9/07/19
Yuma-2	South China	Dual	9/07/19
Si-1	South China	Dual	9/09/19

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

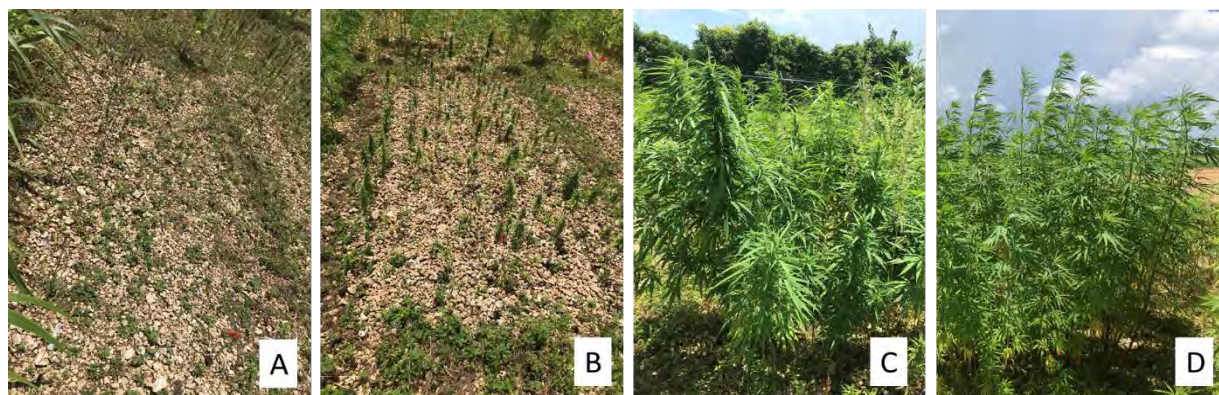


Figure 1. Four hemp varieties planted at TREC May 22, 2019 photographed August 8, 2019. A) Canadian hemp B) European hemp C) Middle Chinese hemp D) South Chinese hemp

Cannabinoid concentration

Approximately 10 g of inflorescence and leaf tissue were sampled at harvest from the main stem of each variety approximately 100 days after planting. The samples were oven-dried at 158 °F for at least 48 hours. Dried tissue was processed and analyzed by UPLC-MS/MS at the UF College of Pharmacy in Gainesville, FL.

Cannabinoid concentration results were limited to a single batch sampling event taken at harvest. Permitted and compliant hemp harvests at the time of this report require total THC concentration by dry weight to not exceed 0.3%. The variety trial results indicated that producing plants with less than 0.3% total THC at harvest may be problematic in South Florida, especially for unpollinated essential oil crops and for several of the grain and fiber genetics that performed well in the trial (Table 2). Because our trial was conducted in the same field, the essential oil varieties were exposed to pollen from the flowering male hemp plants. Essential oil varieties did not exceed 0.3% total THC, but total CBD also did not accumulate above 6%. Two Chinese and two European varieties harvested for grain had total THC under 0.3%. Han NE contained THC above 0.3%, which is notable because it had the highest seed yield of all tested grain varieties. All fiber varieties grown to harvest were Chinese and exceeded THC of 0.3%, most expressing cannabinoid profiles that were dominant in THC.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Table 2. Varieties remaining viable at the end of the growing season sampled for cannabinoid concentration [%]. Average total THC and average total CBD are represented as a batch value for a single sampling event. Total THC values above 0.3% are bolded.

Variety	Origin	Purpose	Total THC [%]	Total CBD [%]
Berry Blossom	USA	Essential Oil	0.229	3.569
Cherry Blossom x T1	USA	Essential Oil	0.261	4.196
Puma-4	South China	Fiber	0.433	0.903
Puma-3	South China	Fiber	0.715	0.674
Yuma-2	South China	Fiber	0.718	0.448
Bama	South China	Fiber	0.839	0.59
Si-1	South China	Fiber	0.943	< 0.05
Han FN-Q	North China	Grain	0.054	< 0.05
Han NW	Central China	Grain	0.152	< 0.05
Carmagnola Selezionata	Italy	Grain	0.169	1.421
Eletta Campana	Italy	Grain	0.195	2.773
Han NE	Central China	Grain	0.527	< 0.05

Planting date trial

Methods

The planting date trial was planted in 2019 consistently across four dates spaced by approximately 3 weeks: May 1, May 22, June 21, and July 18. Trials were designed with consistent plot size, seeding rates, fertilizer rates, and management as per the variety trial methods described above. Eight hemp varieties were selected among available varieties to represent the span of growing purposes (fiber, grain/dual, and essential oil) and region of origin (North America, Europe, and Asia). Stand establishment and yield for fiber and grain producers were evaluated to assess performance of the hemp varieties across planting dates.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Stand establishment

Stand establishment was determined by counting each individual plant within each plot and dividing that value by the number of live seeds planted into the plot. Stand establishment across the planting date trial was relatively low (Fig 2). This should not be an indication of seed quality as seeding rates were adjusted to live seed based on germination tests conducted prior to planting for each variety. Germination tests were generally greater than 80%. Stand establishment declined with later plantings across most varieties. The May 1 and May 22 plantings may represent an important seasonal shift for South Florida as the start of the hot rainy season tends to be mid-May. Heavy rain and flooding may have contributed to poor stand establishment in later plantings. Challenges to plant growth were also observed with plants established in later plantings.

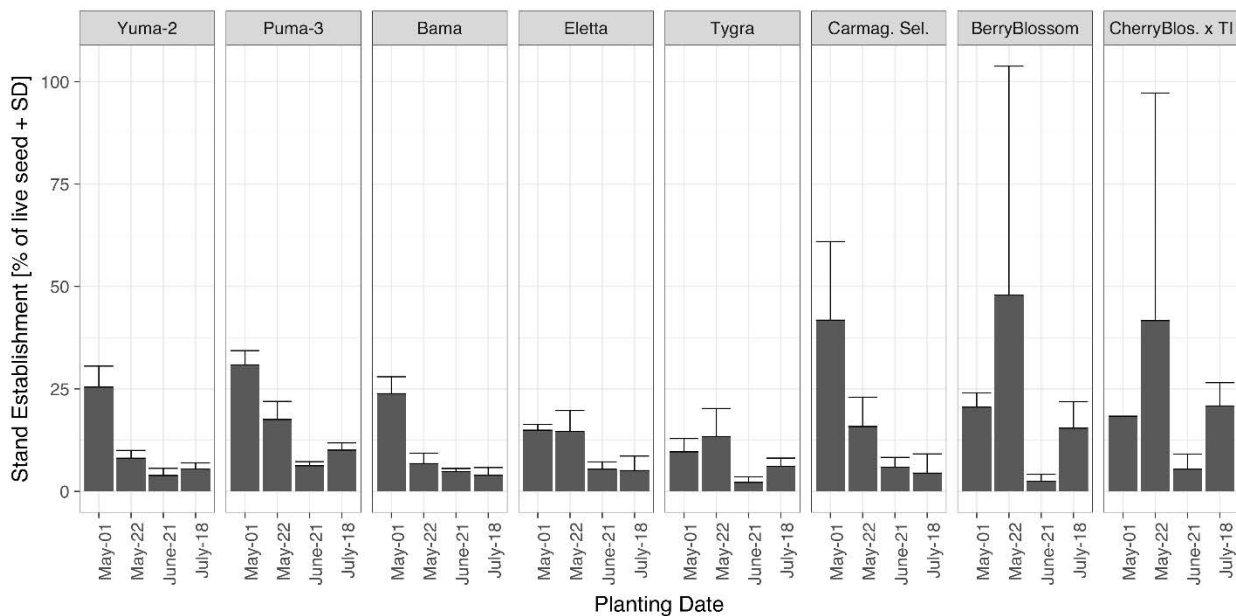


Figure 2. Stand establishment for varieties across planting date in 2019 at TREC as determined by percentage of live seed planted. Error bars represent one standard deviation of the mean.

Fiber and grain yield

Yield measurements were conducted on plants from the first planting of the planting date trial (May 1) and the variety trial (May 22) through August and September at the date each respective variety was ready for harvest. Harvest corresponded to approximately 90-120 days after planting. For fiber crops, stems were harvested at 50% flowering induction for the plot. For grain crops, seeds were harvested from plants that had at least 50% seeds hardened. For dual varieties, the age of plants when they flowered determined if they would be harvested for grain or fiber. Varieties that flowered in August or September developed no seed and were harvested as fiber, while varieties which flowered midsummer and developed seeds during the growing season were harvested as grain. The harvest was taken from a 10.76 ft² area defined as a 3 ft section of the 4 middle rows of the plot.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Fiber yield is reported as dry straw which was the weight of dried stem tissue. To measure dry straw, plants were cut at ground level from the harvest zone and manually defoliated. Fresh stems were weighed, and then a sample was oven-dried at 158 °F for at least 48 hours. Dry weight was recorded for moisture content estimation and yield conversion. Target dry straw weight for commercial fiber production is about 8,000-10,000 lbs/ac. Multiple varieties met that production goal on average, including Bama, Puma-3, Puma-4, Si-1, and Yuma-2 (Fig 3). However, individual stem diameter may be larger than desirable by processing specifications.

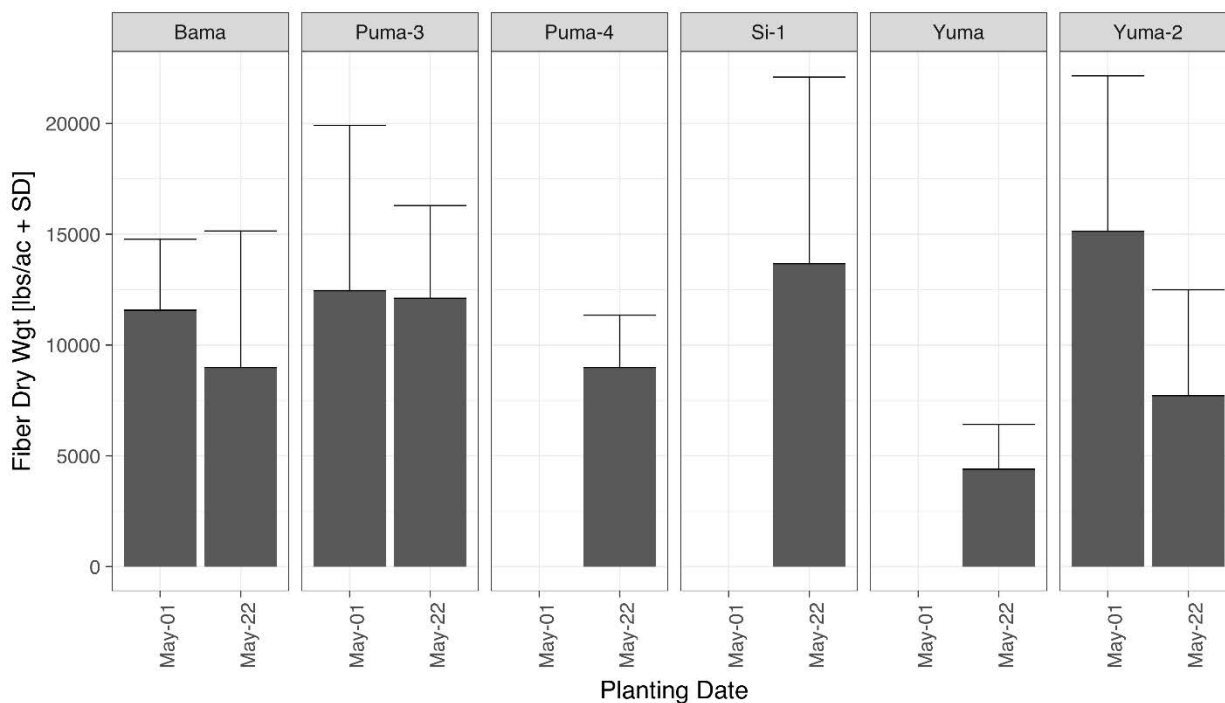


Figure 3. Harvested dry straw weight for varieties selected across planting date in 2019 at TREC as lbs/ac. Error bars represent one standard deviation of the mean.

Grain harvest is reported as total weight of dried seed from the harvested zone. Mature plants were collected and threshed using a small bundle thresher. Fresh grain was weighed and then oven-dried at 158 °F for at least 48 hours for moisture content estimation and yield conversion. Target dry seed weight for commercial grain production is about 800 lbs/ac. Several varieties met that production goal on average, including Carmagnola Selezionata, Eletta, Han NE and Han NW (Fig 4).

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

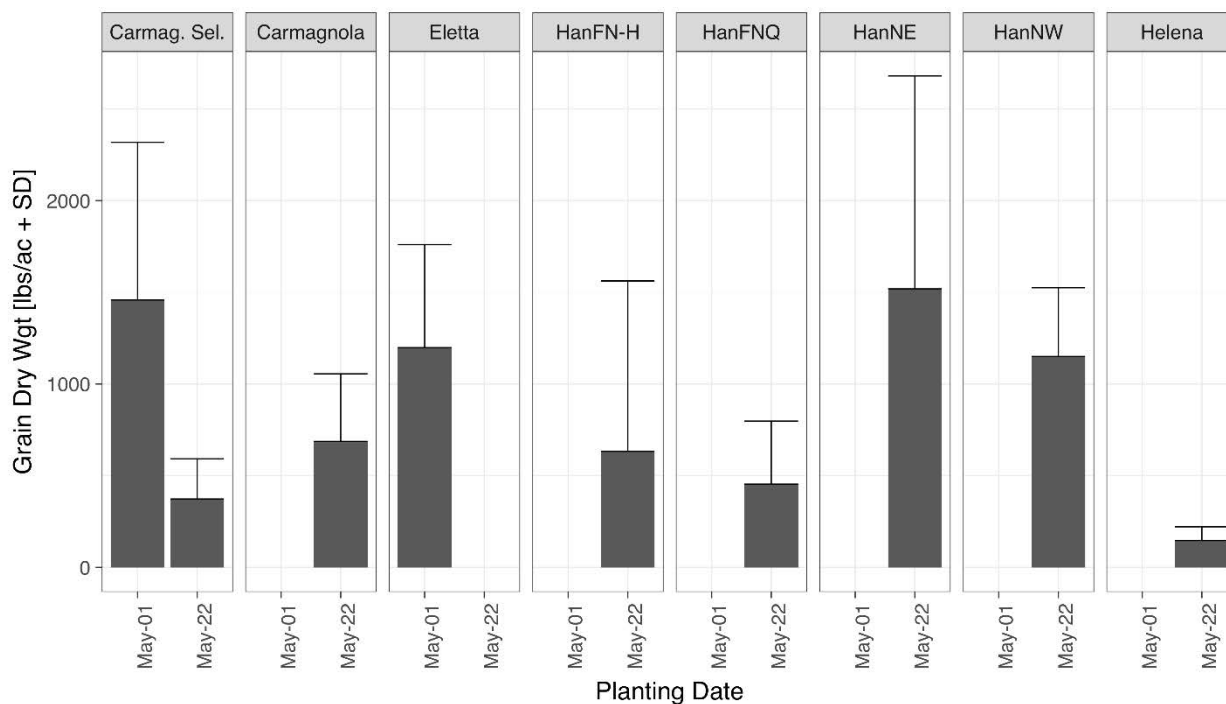


Figure 4. Harvested grain dry weight for varieties selected across planting date in 2019 at TREC as lbs/ac. Error bars represent one standard deviation of the mean.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Preliminary Research Report

Rui Yang

Post-Doctoral
Associate, University
of Florida, North
Florida Research and
Education Center

Josh Freeman

Associate Professor
of Horticultural
Sciences, University
of Florida, North
Florida Research and
Education Center

Erin Berthold

Graduate Research
Assistant, University
of Florida, College of
Pharmacy

Sarah Benevenute

Graduate Research
Assistant, University
of Florida, North
Florida Research and
Education Center

Introduction

Industrial hemp (IH) is *Cannabis sativa* with less than 0.3 % tetrahydrocannabinol (THC) per dry weight. It is a potential new crop for the state of Florida so there are many unknowns regarding its production. The most basic agronomic questions including planting date, planting density, appropriate varieties, soil fertility, and harvest date must be determined to achieve successful production. Most IH varieties are sensitive to day length, when days are long the plant grows vegetatively and when days begin to shorten the plant enters a reproductive phase. There are a multitude of uses for IH which include: fiber, grain for human and animal consumption, and the production of essential oils. Research was conducted during the 2019 growing season at the UF/IFAS North Florida Research and Education Center (NFREC) in Quincy, Florida on IH varieties for essential oil production. These oils are extracted primarily from unpollinated female flowers and contain compounds of interest such as cannabidiol (CBD) and cannabigerol (CBG), all known as cannabinoids. IH is predominately a dioecious plant, meaning it typically has separate male and female plants, but monoecious plants (male and female flowers on the same plant) also exist. Female plants can be obtained by rooting vegetative cuttings from known female plants or by planting feminized seed. At harvest, female flowers are removed, dried and processed to extract the oils.

It should be noted that the research presented in this document was obtained from a single field season and from a limited number of IH varieties. There will certainly be variation between seasons, locations, and IH varieties. **The mention of variety names in this document is not meant to serve as an endorsement nor are these data to be considered a recommendation.**

Objective

To evaluate industrial hemp varieties and management practices to determine which may be suitable for production in northern Florida.

Methods

For all field experiments, feminized seeds were germinated and grown in a greenhouse with supplemental lighting using peat based potting media. Uniform seedlings were transplanted to the field at around 21 days after seeding. All experiments were produced utilizing raised beds and the plasticulture production system that is typical for many vegetable crops. Raised beds were 8 in tall and 30 in wide and were covered with white colored plastic to reduce the soil temperature. Irrigation was provided to the crop through drip tubing located under the plastic.

Variety trial

For any crop, the appropriate variety is a critical factor in achieving successful production. This article only presents flower yield and cannabinoid content which are just a few factors that should be considered in choosing an IH variety for essential oil production. There are also two planting dates presented here. In these experiments, day-length sensitive varieties showed the beginning of flowering around August 7th (day length ~13 h 27 m). This date may not represent a critical day length for all IH varieties.

Three day-length sensitive varieties, including Cherry Blossom (CBL), Cherry×T1 (CT1), and Cherry Wine (CW), were planted on July 3 and 25, 2019, while two day-length neutral (often referred to as auto flower) varieties, including Kayagene 9201 (KG9201) and Kayagene 9202 (KG9202) were planted on July 3 and September 11, 2019.

Spacing between beds was 6 ft and between plants was 5 ft. This row and plant spacing results in 1450 plants per acre.

Fertilizer (N-P₂O₅-K₂O: 10-10-10) was applied under the plastic prior to planting and soluble fertilizer was delivered through the irrigation system during the growing season. Total fertilizer application for the season was 150, 100, and 200 lb/acre for N, P₂O₅, and K₂O, respectively.

Plants were harvested at maturity, dried in a forced-air drier at 130 °F for 72 h, flowers were removed by hand and flower yield was recorded. Flowers were then ground into fine powder using a small coffee grinder for cannabinoid analysis. USDA allows a measurement of “uncertainty” (analytical error) in addition to the result. The analytical method used in our study has an uncertainty of 0.05%, therefore, the THC threshold of 0.35% was used in the following comparison. It should be noted that final rules for hemp production have not been set at the time this article was written.

Different lowercase letters indicate differences among varieties for each planting date.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Day-length sensitive varieties

Overall, the 1st planting date had greater flower yield relative to the 2nd planting date. Cannabinoid concentrations were not significantly different between the two planting dates.

Variety	Flower yield (lb/acre)	THC	CBD	CBG
		% dry weight		
Planting date July 3, 2019 – Harvest September 26				
CBL	2730 a	0.521 ab	9.589 a	0.197 ab
CT1	2424 b	0.582 a	10.254 a	0.260 a
CW	2352 b	0.474 b	8.927 a	0.189 b
Planting date July 25, 2019 – Harvest October 17				
CBL	1326 ab	0.502 ab	9.477 a	0.208 a
CT1	1467 a	0.607 a	10.923 a	0.246 a
CW	703 b	0.473 b	8.895 a	0.201 a

Day-length neutral varieties

The two day-length neutral varieties had significantly lower flower yield and cannabinoids compared to the day-sensitive varieties.

Unlike the day-length sensitive varieties, flower yield did not differ between the two planting dates of the day-length neutral varieties.

Variety	Flower yield (lb/acre)	THC	CBD	CBG
		% dry weight		
Planting date July 3, 2019 – Harvest August 22				
KG 9201	66 a	0.28 a	4.54 a	0.20 a
KG 9202	149 a	0.31 a	5.56 a	0.21 a
Planting date September 11, 2019 – Harvest November 6				
KG 9201	77 a	0.33 a	6.28 a	0.22 b
KG 9202	100 a	0.38 a	7.30 a	0.32 a

All day-length sensitive and day-length neutral varieties evaluated at NFREC in 2019 tested above the THC threshold at harvest except for KG 9201 on the July 3rd planting date.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

Plant density study

Plant density or plant population is another critical factor that can have tremendous impact on crop yield per acre and therefore profitability. This article only presents flower yield and cannabinoid content which are just a few factors that should be considered in choosing an IH planting density.

Two of the day-length sensitive varieties, CT1 and CW, were evaluated using the same field setup as the variety trial, except for the plant densities. Four different plant densities were achieved (1210, 1613, 2420, and 4840 plants per acre) by using different in-row spacing (1.5, 3.0, 4.5, and 6.0 ft).

There was no interaction between IH variety and plant density, so the data were combined to demonstrate the impact of plant density. Different lowercase letters indicate differences among plant densities.

On a per plant basis flower yield gradually increased as the plant density decreased (less plants per acre led to more flower yield per plant). However, this trend was reversed when flower yield was considered on a per acre basis. Flower yield per acre increased as plant density increased (more plants per acre increased flower yield per acre). Cannabinoids did not significantly differ among plant densities. It should be noted that at harvest the average THC content of these two varieties was above 0.35% per dry weight. It should be noted that this study was carried out on one planting date. Because IH is sensitive to day length, any change to an earlier or later planting date may impact the results.

Plant density (plants/acre)	Flower yield (lb/plant)	Flower yield (lb/acre)	THC	CBD	CBG
			% dry weight		
4840	0.85 b	4288 a	0.56 a	11.82 a	0.24 a
2420	1.34 a	3256 b	0.54 a	11.78 a	0.22 a
1613	1.58 a	2452 c	0.58 a	11.99 a	0.23 a
1210	1.69 a	2056 c	0.58 a	11.96 a	0.22 a

Cannabinoid development

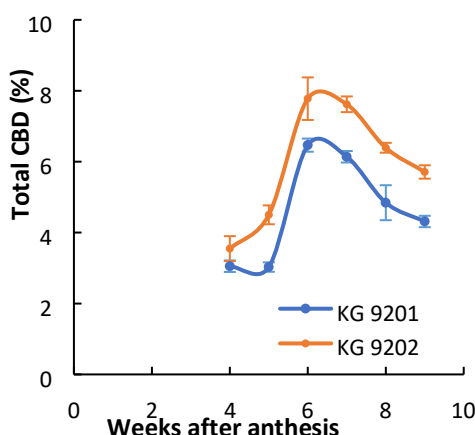
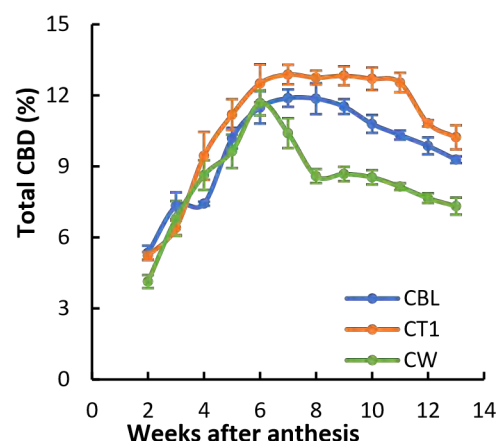
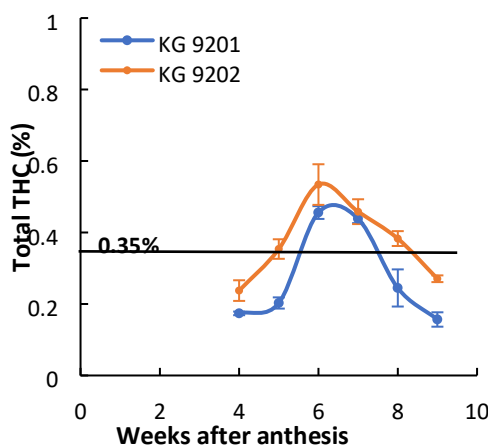
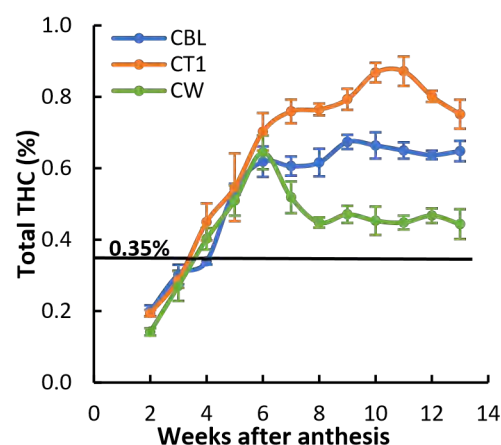
One potential use and market for IH is the production of essential oils. These oils contain many compounds including several cannabinoids. A few of these are cannabidiol (CBD) and cannabigerol (CBG). For this market, the value of the crop is determined by the cannabinoid content in the flowers. Therefore, in order to maximize profit, it would be critical to harvest flowers when these compounds are at or near their maximum concentrations. Another critical factor to consider in these data is the THC concentration in female flowers. To be legally considered industrial hemp, the THC content must be below $0.3 \pm 0.05\%$ per dry weight (dependent on final USDA and Florida Department of Agriculture and Consumer Services rules).

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

The same field setup as the variety trial was adopted for this study. Flower samples were taken from the top 1/3 of 5 uniform plants within a plot on a weekly basis from 2-4 weeks after anthesis (beginning of flowering) until full senescence of the plants. Flower samples were dried in an oven at 130 °F for 72 h and ground for cannabinoid analysis.

Total THC

A significant difference was observed between varieties. For CW, the THC content in flowers gradually increased, sharply dropped after reaching a peak, and remained relatively consistent as flowers continued to age. For the CBL and CT1, the THC content remained relatively consistent after reaching the peak. For all the three varieties, THC went above threshold at 4 weeks after anthesis and stayed above threshold for the rest of the season. For the two day-length neutral varieties, the change of THC content in flowers increased quickly and then dropped below the threshold at 8 weeks after anthesis.



Total CBD

The change in CBD concentration approximately synchronized with THC concentration. After CBD content reached the peak, the CT1 and CBL had a plateau stage of about 6 and 4 weeks,

For more information visit: <https://programs.ifas.ufl.edu/hemp/>

respectively, whereas the CBD content sharply dropped for CW and the two day-length neutral varieties.

A change in 2-3% in CBD content could make a significant difference in the profitability of IH so it is important for producers to track the CBD concentration during the season. These data also illustrate the potential of the varieties tested to rapidly go above the THC threshold and for the day-sensitive varieties, to remain above the threshold for the rest of the season. Based on proposed regulations, this scenario could render the crop unmarketable. It is unclear if this trend will be repeatable in other seasons but certainly THC concentration should be closely monitored in an IH crop to avoid undesirable outcomes for the crop. It should be noted that the CBD/THC curve data presented here is based only on flower samples with no leaf and stem included. The final rules for sampling may require the top 8" of flower or the top 1/3rd of a plant which would include some stem and leaf. This could marginally reduce the CBD and THC content compared to what is presented here.

For more information visit: <https://programs.ifas.ufl.edu/hemp/>



Phenotyping Commercial Varieties of Industrial Hemp (*Cannabis sativa* L.) for Disease, Maturity, and Yield in the Southeastern U.S.

Maryjo Valle¹, John Erickson¹, Zachary T. Brym², William Wadlington², Esteban Rios¹, Josh Freeman³
¹ Agronomy Department, University of Florida, Gainesville, FL, USA, ² Tropical Research and Education Center, University of Florida, Homestead, FL, USA,
³ North Florida Research and Education Center, University of Florida, Quincy, FL



Background and Objectives

With recent changes in the 2018 U.S. Farm Bill, there is growing national interest in the production of industrial hemp (*Cannabis sativa* L.) for fiber, seed, and CBD oil. However, there is limited information on the performance of current varieties in the U.S., especially in humid sub-tropical, low latitude environments. Thus, the objective of this study was to phenotype 24, mostly international, fiber, grain, and CBD industrial hemp varieties in North Central Florida for phenology, yield, and disease tolerance to identify germplasm that is well adapted for production in the North Central Florida environment. Five separate field trials were established in Hague, FL to gauge hemp performance in Spodosol soils, the most extensive soil type in the state, found in 1.5 million acres across Florida (USDA NRCS). The crop's phenology was evaluated from May to August 2019 and disease incidence/tolerance was noted.



Figure 1. A) Stand of southern Chinese industrial hemp variety, Yuma-2, first planted on 5/7/19. B) Same stand on 6/12/19 fertilized with 150 kg N ha⁻¹.

Materials and Methods

The population was composed of: i) 17 grain and dual fiber/grain varieties; ii) 4 fiber varieties; and iii) 3 high CBD oil varieties. Experiments were established in the summer of 2019 in Hague, FL as a randomized complete block design with four replicates per variety. Plants were sown into a 6 ft by 10 ft plot with an 8 in. row spacing and 1/2 inch seeding depth for a sowing density of 100 seed per 10 foot row. The population was evaluated from May to August 2019 for the following traits: germination, flowering time/set, height, biomass, yield, and disease incidence/tolerance. Data was analyzed in R.

Field Trials

1. Variety Trial (Grain/dual, fiber, CBD)
 - Identify germplasm well adapted for production in Florida.
2. Nitrogen Fertility Trial
 - Identify optimum nitrogen fertilizer range for seeded hemp.
3. Plant Density Trial
 - Identify effects of planting date on growth, yield, and phenology
4. Bed Trial
 - Address excessive soil moisture and flooding
5. Pre-emergent Herbicide Trial
 - Address increased weed suppression in field trials.

Results

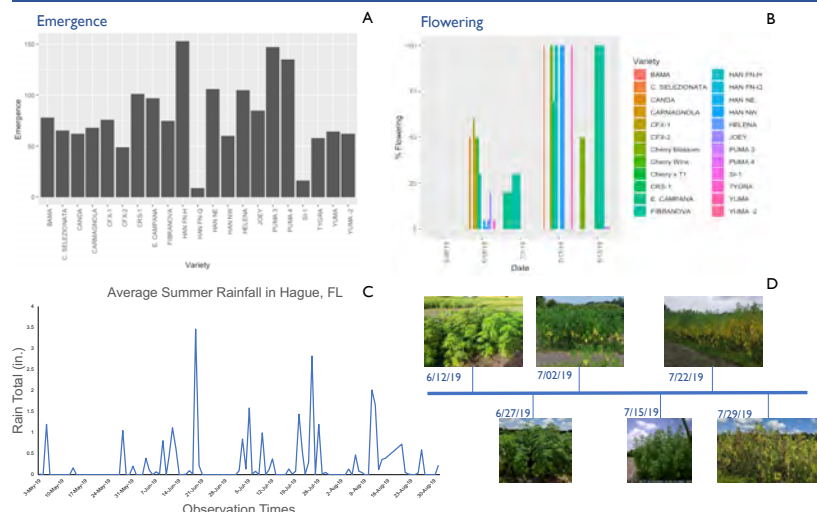


Figure 2. A) Emergence of dual/grain and fiber varieties sown on 5/28/19. B) Flowering percentages of hemp varieties throughout the summer. C) Average summer rainfall in Hague, FL. D) Timeline of Yuma 2's decline in plant health due to torrential rains.

All Canadian and European hemp varieties (i.e. C. selecionata, Canda) started flowering three weeks after planting and had low to moderate emergence (Figure 2 A and B). By 7/17/19 more than 75% of plants within a plot had flowered. Southern Chinese varieties had low to moderate emergence, but did not flower throughout the summer months. Our field trials faced multiple production challenges, such as disease, excess soil moisture, and increased weed competition. Torrential rains during the summer severely impacted the performance of hemp varieties that exacerbated their growth. All of our plantings experienced excessive soil moisture as young plants and the poor drainage of the site stunted their growth and survival. Figure D follows Yuma-2's quick decline in health after sequential rain in July.

Current Work



Figure 3. A) A CRD design of 42 Yuma-2 plants undergoing various frequency and flooding durations to mimic the conditions observed in the field. B) Yuma-2 undergoing weekly flooding for 24 hours. C) Yuma-2 undergoing weekly flooding for 96 hours. D) Yuma-2 treated with 0.25% colchicine for 12 hours.

Flood Stress

Based on the poor performance of industrial hemp in waterlogged soils in Hague, Florida, a separate greenhouse study has been developed to determine the maximum water stress conditions hemp can withstand. The objective of this study is to evaluate the effects of flood duration (0 h, 24 h, 48 h, and 96 h) and frequency (weekly and biweekly) on industrial hemp variety Yuma-2 survival, growth, and assimilate partitioning (Figure 3 A-C).

Ploidy

Efforts to double diploid industrial hemp is underway by submerging hemp seedlings in varied colchicine concentrations (0.1%, 0.25%, 0.5%) for 6 h, 12 h, or 24 hrs. Successful development of tetraploids will allow us to evaluate their performance compared to diploid hemp (Figure D).

REFERENCE: U.S. Department of Agriculture, Natural Resources Conservation Service Florida: Soils. By Kevin Sullivan. Available at: <https://www.nrcs.usda.gov/wps/portal/nrcs/main/fl/soils/>



UF/IFAS Industrial Hemp Pilot Project – 2020 Hemp Industry Insights Presentation

INSIGHTS INTO BUILDING A HEMP INDUSTRY IN FLORIDA

**UF/IFAS Tropical Research and Education Center
Homestead, Florida**

**Dr. Trent Blare
Assistant Professor**

**Fredy Ballen
Data Management Analyst**

**Martha Rivera
Research Assistant**

Presentation Outline

- I. Introduction and objective
- II. Hemp Production Systems and Applications
- III. Hemp Industry in the US
 - i. Retail Market and Imports
 - ii. Hemp Production in the US and Florida
- IV. Risks and Challenges in the Hemp Industry
- V. CBD Regulatory Issues
- VI. What the Industry Needs

For more information visit programs.ifas.ufl.edu/hemp

CONTEXT

- Need for alternative cash crops:
 - Disease and pests – citrus and avocados
 - Foreign competition -tomatoes
 - Natural disasters
- Industrial hemp legalized in 2018
- Notable successes for early adopters
- Continued challenges:
 - What are the best management practices?
 - Is hemp profitable?
 - What regulations would best support the industry?

OBJECTIVES

- Examine the hemp industry in the United States
- Analyze the potential for hemp among Floridan farmers, processors/ manufacturers, and consumers



Parts of the hemp plant and their uses

- Grain / seed
- Fiber
- Flower/biomass (cannabinoids)

HEMP PRODUCTS



Source: manitobaharvest.com, vitacost.com



Source: Patagonia.com



Hemp Hurds & Shives



Hempcrete Blocks



Hempcrete Panels



Hemp Fiber Ball Insulation



Source: cbdnetwork.com

Source: Hempcrete Direct

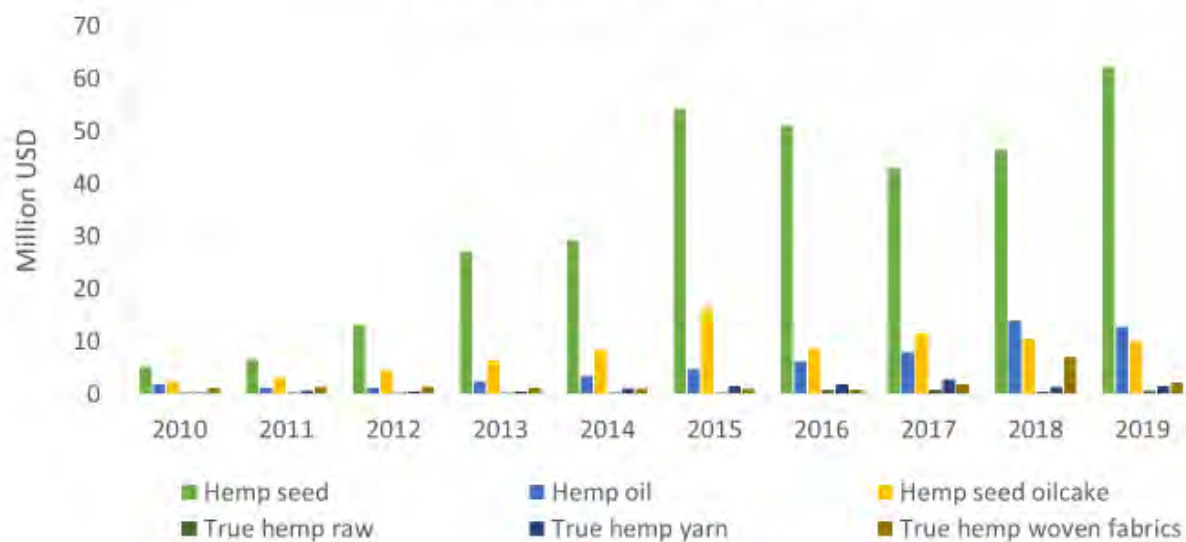
Source: Hempcrete Direct

US HEMP INDUSTRIES



U.S. retail market for hemp-derived products. Source: Hemp Business Journal

US HEMP IMPORTS



Source: USOA GATS 2020

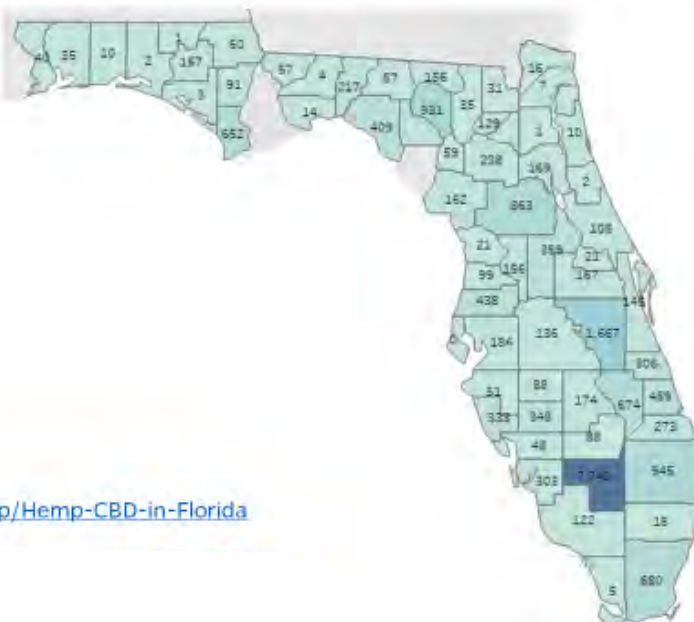
LICENSED ACREAGE IN THE US

						% Share
State	2016	2017	2018	2019	2020	2018-20
Colorado	5921	9700	21578	87359	62208	15.37%
Kentucky	2525	3271	6700	60000	32106	8.87%
Oregon	500	3469	7808	63000	29772	9.03%
Tennessee	225	200	3338	45480	51000	8.97%
Montana		542	22000	45000	11688	7.07%
Others	599	8531	16752	268518	279049	50.69%
Total	9770	25713	78176	569357	465823	100.00%

Source: Mota Hemp, Hemp Benchmarks, Hemp Industry Daily

STATE OF FLORIDA

- 610 growers
- 13 processors
- 21,708 licensed acres
- Top counties:
 - Hendry ~ 7,740 acres
 - Osceola ~ 1,670 acres
 - Palm Beach ~ 950 acres



*As of October 2020

Visit: <https://www.fdacs.gov/Cannabis-Hemp/Hemp-CBD-in-Florida>

RISKS AND CHALLENGES

- Little to no experience growing hemp
- Limited access to certified seed and/or pilot-project approved varieties
- High costs, especially for CBD hemp (seed/transplants, testing, labor, security, etc.)
- Limited access to capital

RISKS AND CHALLENGES

- Testing for THC
 - Must be done within 15 (soon 30) days prior to harvest
 - 5 approved labs for the state of FL
 - “hot” hemp (> 0.3% total THC) must be destroyed
- Difficulty finding a buyer for your crop
 - Tennessee: 22% of farmers operated under a contract
 - Connecticut: 32% of farmers sold directly to consumers, 32% to a processor/manufacturer and 36% did not find a market
 - Florida has 13 processors in the entire state

CBD

- FDA has not released CBD regulations
- “It is currently illegal to market CBD by adding it to a food or labeling it as a dietary supplement.” – FDA
- No quality standards
- No labeling requirements
- No dosage specifications, how much is enough? How much is too much?
- Will CBD and other cannabinoids be regulated as a pharmaceutical? Over-the counter? Prescription?

WHAT THE INDUSTRY NEEDS

- Education
 - Access to information: online, workshops, webinars, conferences
 - Erase the stigma: law enforcement and policy makers
 - Legal advice to farmers: inspections procedures, THC test is above the legal limit
- Access to credit
- Clarity and coordination among state and federal regulations
 - Harmonize CBD regulations across all states
 - Clear transportation rules

What is needed for a successful Hemp industry...

- Consistent and accurate data/metrics
 - Planted/harvested acreage
 - Pricing
- More research
 - Attributes of different varieties
 - Crop management systems
 - Drying and storage practices
 - Financial viability and marketing
 - Alternative applications for hemp
 - Accurate THC testing methods
 - Environmental Impacts



Source: UF/IFAS, TREC

Thank you!

Please note...

- Hemp economics EDIS document coming soon (Summer/Fall 2021)
- Peer-reviewed publication on viability of a Florida hemp industry (Late 2021)

Is a Viable Hemp Industry in Florida's Future?

Trent Blare, Martha Rivera, Fredy Ballen, Zachary Brym

**University of Florida Institute of Food and Agricultural Sciences
Tropical Research and Education Center
Homestead, FL USA**

Dr. Trent Blare, Assistant Professor
UF/IFAS Food & Resource Economics
Email: tblare@ufl.edu

Industrial Hemp Pilot Project: Invasion Risk

Susan Canavan, Zachary Brym, Daniel Calzadilla, Kristin Hinkson,
S. Luke Flory

Summary

- *Cannabis sativa*, including varieties of hemp, can become a problematic invasive species and a weed of agriculture when not properly managed
- A recent evaluation by the UF/IFAS Assessment of Non-native Plants in Florida's Natural Areas found that hemp is a 'high invasion risk' for Florida
- We conducted field experiments to help predict high risk conditions for hemp establishment and persistence outside of cultivation in Florida
- Preliminary results show that hemp may establish outside of cultivation and produce volunteer crops
- Optimal conditions for establishment include dispersal of many seeds, highly disturbed soils, and open habitats

Background

Invasions of non-native plant species are a significant ecological and economic problem for the state of Florida. More than 1500 non-native plant species are documented as established in the state, and about 10% of these have invaded natural areas (Colunga-Garcia et al. 2013). Although a fraction of non-native species are problematic, invasions in natural areas cause severe economic and environmental damage. Invasive species have significant impacts on the state's agriculture, tourism, and recreation industries, and more than \$45 million is spent per year to manage plant invasions in Florida's natural areas (Hiatt et al. 2019).

There are increasing concerns about the invasion risk of hemp, especially in areas where cannabis production has only recently been initiated, such as Florida. Cannabis (including hemp and other varieties) has a known crop-to-weed history, a tendency to escape cultivation (Small et al. 2003; USDA and NRCS 2020), and may become an invasive species in natural areas and a weed of agriculture. A recent evaluation by the UF/IFAS Assessment of Non-native Plants in Florida's Natural Areas (assessment.ifas.ufl.edu) found

that hemp is a 'high invasion risk' for Florida, primarily because of its biological characteristics and because it has escaped and colonized natural areas in other states and countries.

There have been observational notes and a few scattered studies on specific factors that facilitate hemp escape and invasion. However, more information is needed to determine the combination of conditions that are conducive for establishment and persistence of cannabis outside of cultivated areas including differences among hemp biotypes, and the effects of soil disturbance and nutrient availability.

Objective

To better understand the biotic and abiotic factors that contribute to hemp establishment and persistence (i.e. surviving and producing viable seeds) outside of cultivation.

Research

Establishment Experiment: A field experiment was undertaken at the Bivens Arm Research Site (BARS), Gainesville, FL, that was based on "what if" scenarios where we introduced hemp under realistic conditions that might be expected if seeds were left in an agricultural field, lost during transport, or dispersed to a natural area (Fig 1A). A randomized block design was used to test how germination rates and persistence of hemp vary across three biotypes ('CFX-1', 'Si-1', 'Fibranova'), disturbance regimes (low, medium, or high), and levels of propagule pressure (50, 250 or 1000 seeds added to a plot), which were then nested within three different habitats (open-field, forest edge, or forest). The experiment initiated on the 14th June 2019 and was ended about four months later when most plants had senesced. For the initial emergence of seeds, the different treatments influenced the number of seeds that emerged (e.g. plots with higher disturbance had more seeds emerging) (Fig 1A). However, many plants died within a few weeks, likely because of very low rainfall, leading to very few established plants.

Multi-site Experiment: A second experiment was completed in 2020 at two additional sites. The emergence and persistence of hemp was tested under high soil disturbance (tilled) and low soil disturbance (not tilled at time of experiment) (Fig 1B-C), different nutrient levels (no fertiliser or fertiliser added in amounts expected in typical hemp cultivation) and with different biotypes ('CFX-1', 'Han-NE', 'Helena'). The experiment was conducted in southern Florida (Tropical Research and Education Centre-TREC, Homestead, FL) and in northern Florida (Plant Science Research and Education Unit- PSREU, Citra, FL). The southern site had considerably more emergence and establishment of plants than the northern site (~26 x more plants). For emergence and establishment, disturbance was a significant factor, in that more disturbed plots had more hemp plants. Biotype and nutrients had mixed effects depending on the site, for persistence nutrients promoted the growth of other vegetation reducing the success of the hemp. The specific site environmental factors (soil

type, climate etc.) appeared to have a large effect on hemp success with much more establishment at the southern site.

Germination Experiment: Germination experiments were conducted in April 2019, and repeated in March 2020 with the same stock of seeds. Seeds had been stored in a facility with an average temperature of 19.5°C (+/- 1°C). Twenty seeds of each biotype (x 5 replications) were germinated in Petri dishes in growth chambers at 26°C (optimal germination temperature for hemp). There was a considerable decline in the viability of seeds (% of seed that germinated) over one year suggesting that the longevity of a seed bank might be limited. (Fig 2A). Other seeds metrics were also taken for experimental planting, such as seed weight (Fig 2B). The difference in seed traits, such as weight, likely affects seed dormancy and germination across biotypes.

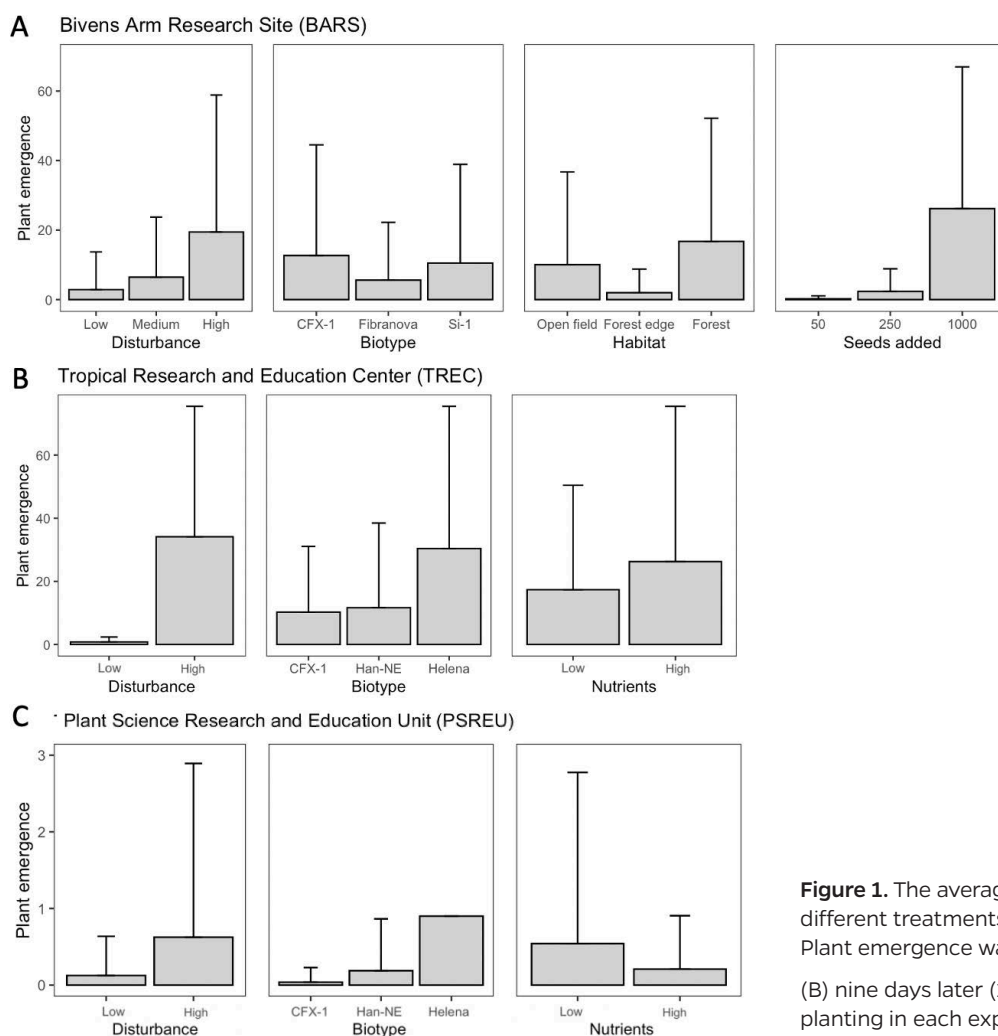


Figure 1. The average emergence of hemp seeds per plot, across different treatments for three experiments in three different sites. Plant emergence was (A) seven days (405 plots) (B) nine days later (120 plots) and (C) four days (120 plots) after planting in each experiment.

Preliminary key points

- As expected, high disturbance was consistently an important factor for the initial establishment of hemp across all experiments. If seeds were to escape beyond cultivated conditions they would have a higher likelihood of establishing in disturbed areas such as roadsides, ditches, and abandoned fields.
- Greater soil nutrients (i.e. fertilizer) had a mixed effect on hemp. Although hemp does well with high levels of nutrients initially (and in a cultivated setting), in an abandonment situation where other competing plants are present they can suppress hemp and reduce establishment.
- There are important physiological and morphological differences among hemp biotypes that can affect germination and establishment. Therefore, we expect invasion risk varies to some level across biotypes.
- Hemp seeds rapidly lost viability over time, that seed bank dormancy may not be a significant contributor to invasion risk of cultivated biotypes.

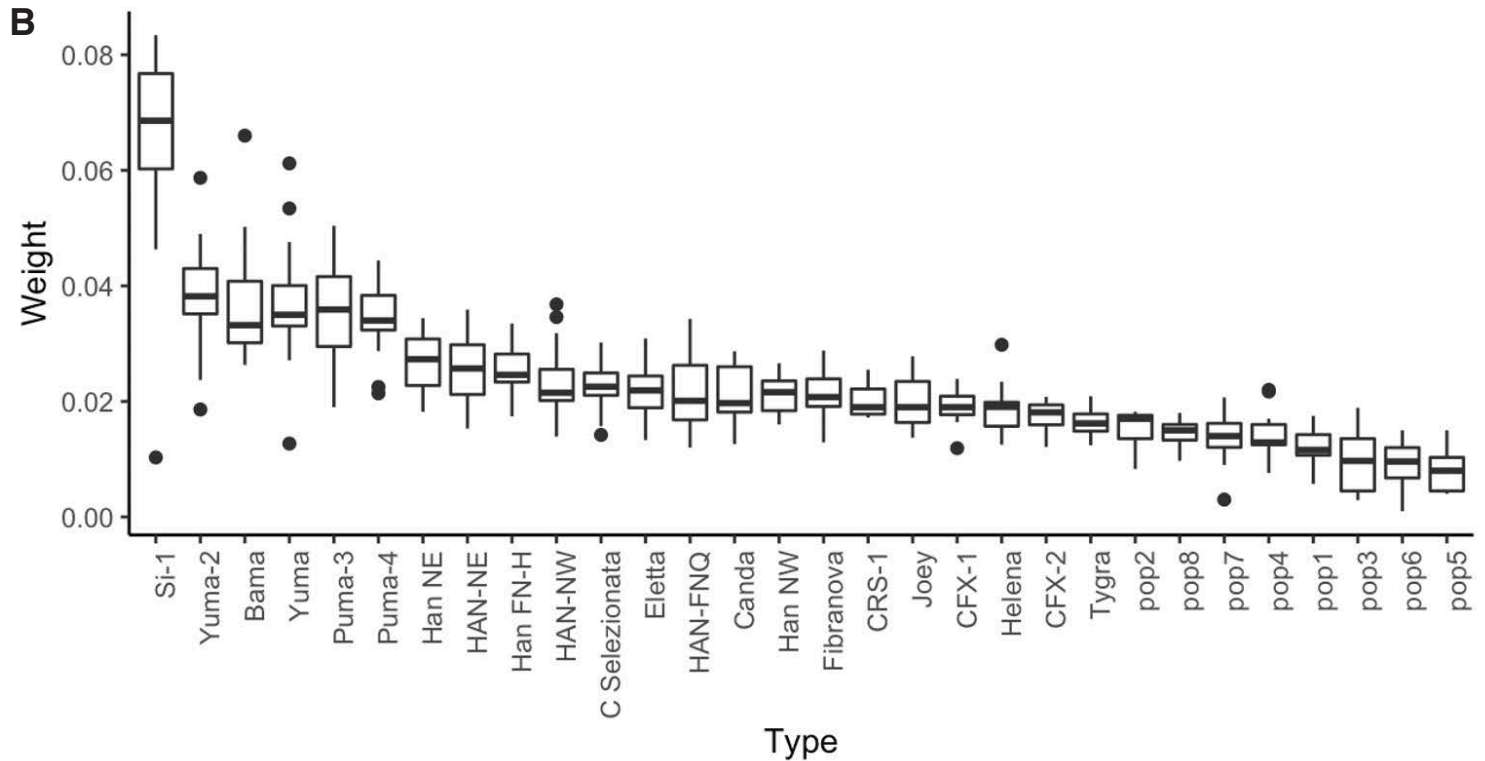
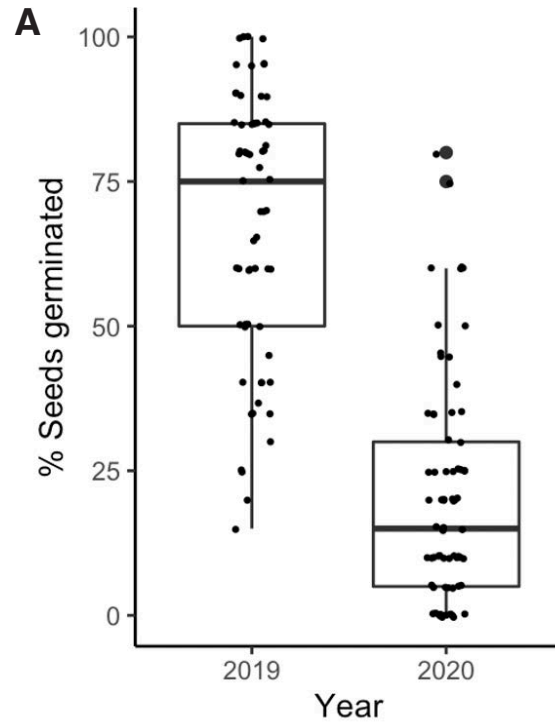


Figure 2. (A) the percent of seeds that germinated in lab trials declined after one year across all biotypes. (B) There was large variation across biotypes of the average seed weight (g).



Figure 3. Selection of field experiment photographs. (A-D) the initial set-up of the invasion risk experiment at BARS, Gainesville, FL. (A) Plots were hand tilled to replicated three different levels of disturbance, seeds were broadcasted on top to replicate spilled or escaped seeds. (B) Dampingoff and snail herbivory were seen in plots, especially those located in the forest and forest-edge, reducing the persistence of seedlings. (C-D) Hemp plants that did emerge, had to compete with other vegetation as the plots were not interfered with in order to recreate a typical level of competition that escaped plants would have to contend with. (E-F) A second experiment was done at a different location at the Plant Science Research and Education Unit- PSREU, Citra, FL. (E) Plots were mechanically prepared to have low or high disturbance, and additional nutrients (fertiliser or no fertiliser) was included as a treatment. (F) Plants that did reach maturity were smaller than those seen in cultivation.

References

- Colunga-Garcia M, Haack RA, Magarey RD, Borchert DM (2013). Understanding trade pathways to target biosecurity surveillance. *NeoBiota*, 18, 103–118
- Hiatt D, Serbesoff-King K, Lieurance D, et al (2019) Allocation of invasive plant management expenditures for conservation: Lessons from Florida, USA. *Conserv Sci Pract* 1:e51. <https://doi.org/10.1111/csp2.51>
- Small E, Pocock T, Cavers PB (2003) The biology of Canadian Weeds. 119. *Cannabis sativa* L. *Can J Plant Sci* 83:217–237
- USDA, NRCS (2020) The PLANTS Database: *Cannabis sativa* L. (<https://plants.usda.gov/core/profile?symbol=CASA3>, 6 July 2020). National Plant Data Team. Greenboro, NC 27401-4901, USA

Multi-Disciplinary Public-Private Sector Partnership in Industrial Hemp

Heather Kalamán, Maryjo Valle, Jordan McBreen, Keir Hamilton, Janam Acharya, Esteban Ríos, Md Ali Babar, Amanda Hodges, Gerardo Celis and Diane Rowland¹

Summary

Collaborations between academia and industry have always been groundbreaking as both research entities continue to evolve and have developmental advancements. Although both realms of research have vastly different models, there is a mutual influence between both fields – introduction of new methodologies and knowledge to companies, and exposing students to practical expertise and cooperative projects. The partnership between the University of Florida (UF) Agronomy Department and Syngenta Flowers began in the Spring 2020 with the goal of creating a multi-disciplinary team of students, faculty and researchers to target research, teaching, and extension activities with industrial hemp (*Cannabis sativa* L.). The overall goal was to screen varieties and develop recommendation guidelines that would be adopted by Florida growers. Specifically, the multi-disciplinary team was created to tackle research questions related to hemp production in South Florida, including variety testing, agronomic management and identification of major pests and diseases occurring in the field. Later, a Teams Project class was designed to give

students the opportunity to process, analyze and report data collected during the research project in the Fall 2020, as well as learning professional development skills.

Introduction

The students first developed an extensive literature review in each respective focus area – breeding, agronomy, pests and diseases – to identify potential varieties to use in the study, management practices to maximize production, pests or diseases to scout for and potential effects this could have on cannabinoid sampling.

Accordingly, a field experiment was created to evaluate 21 varieties, both CBD and grain types, in Alva, FL. Experimental design followed a randomized complete block design with four blocks. Plots were established using rooted cuttings on July 29, 2020 and data was collected during the period of August 12th to October 7th every two weeks. Data was collected for several plant characteristics and pest/pathogens according to each student's educational discipline Table 1.

Table 1. Plant characterization and pest/pathogens for the hemp field trial conducted in Alva, FL in 2020.

Study Area	Traits	Description
Agronomy/Breeding	Plant Growth	Plant height and width; Growth Index; Branch number; and Growth Habit
	Chemical Analysis	Composite samples of flowers were collected several times to represent CBD and THC concentrations evolution over time.
	Dry matter yield	Whole plant and flower yield; and Flower/Stem ratio
Pests/Pathogens	Scouting	Scouting for whitefly, <i>Bemisia tabaci</i> Biotype “B”, and the immature leafminer, <i>Liriomyza</i> sp. <i>Heliothis</i> pheromone traps were installed, assessed and replaced every two weeks. Hemp varieties were scouted for disease pressure both pre and post-anthesis. Diseased plant samples notated in the field were submitted for further analysis to the UF Plant Diagnostic Center in Gainesville, Florida.

¹Heather Kalamán, doctoral student, Doctor of Plant Medicine Program; Maryjo Valle, master's student, Agronomy Department; Jordan McBreen, master's student, Agronomy Department; Keir Hamilton, doctoral student, Doctor of Plant Medicine Program; Janam Acharya, master's student, Agronomy Department; Esteban Ríos, assistant professor, Agronomy Department; Md Ali Babar, associate professor, Agronomy Department; Amanda Hodges, associate extension specialist and director of the Doctor of Plant Medicine Program; Gerardo Celis, lecturer Agronomy Department; and Diane Rowland, professor, agronomy department chair, and director for Center for Stress Resilient Agriculture; UF/IFAS Gainesville, 32611.



The teaching component of the collaboration was addressed through the creation of a Project Team Research course (ALS 6031), developed to provide students with real world industry experience by allowing partnerships and collaboration between UF students and staff with representatives of Syngenta Flowers that mentor both collaborative and independent decision-making skills. With this objective in mind, students and industry employees could facilitate the important exchange of ideas and information with the goal of establishing protocols and exploring early data for CBD hemp production in Syngenta Flowers' pilot trials for hemp cutting production. As part of the class students were also trained in data processing and analysis. The final report for this class will contain results and conclusions for the research component of the partnership. Finally, students were exposed to improving their skills in the area of professional development. The Syngenta Flowers provided mentoring in the Project Team Research course in regards to professional development under topics such as: Project and Time management, Marketing and Product management, Cutting – Technical service perspective, Sales, Economics, Breeding, Plant Pathology.

Output

The collaborative model provided students with first-hand experience on the process of commercializing a product i.e., hemp cuttings, starting from team creation and

management, planting and data collection, to sales and distribution. This collaboration allowed for two research entities, Syngenta Flowers and the University of Florida, to rely on each other for their merits. At the end of the collaborative period, several deliverables will be expected of the students on the team. The first being a report that details the entirety of the project from beginning to finish that reads like a typical article one might expect to find in an academic journal. This report will include an introduction to the project and background information on hemp, a section discussing the materials and methods used to carry out the study and collect the data, a section discussing the results from the analysis of the data, and finally a portion discussing what the results mean to us and to industry at large. Secondly, they will deliver a mock Sales Sheet that follows the template laid out by Syngenta Flowers as a quick overview of the potential hemp products, giving the students an opportunity to develop a professional document that is typically used in industry. Finally, the students will present their findings UF Agronomy and Syngenta Flowers collaborative teams in a group presentation where the students will get to explain their methods and results, providing a great opportunity for group effort and public speaking. Results from this study may provide a variety of recommendations for the industry and Florida hemp farmers. The partnership between Syngenta Flowers and the UF Agronomy Department fostered robust research and education to later be utilized by hemp farmers and growers.

Hemp Fertilization: Current Knowledge, Gaps and Efforts in Florida: A 2020 Report¹

Rao Mylavarapu, Zachary Brym, Luis Monserrate, and Michael J. Mulvaney²

Introduction

Starting the last week of April 2020, the Florida Department of Agriculture and Consumer Services began accepting applications for cultivation of hemp (*Cannabis sativa*; Figure 1) in Florida, with the potential for building a \$20–\$30 billion industry in the state.



Figure 1. Hemp (*Cannabis sativa*) cultivation in Florida.
Credits: Luis Monserrate, graduate student, UF/IFAS Agronomy Department

Hemp Usages and Production Systems

Hemp is an annual herbaceous plant that may be grown for fiber, seed, or flowers. However, it is not a cover crop like sunn hemp (a legume, *Crotalaria juncea*; Figure 2) and is

also different from jute (a fiber crop, *Corchorus olitorius*; Figure 3). Hemp is classified as a noxious weed in several states and is predicted by the UF/IFAS Assessment of Non-native Plants in Florida's Natural Areas to have a high invasion risk in Florida. Hemp is predominantly a short-day plant, and the reproductive phase will begin only when the day length is less than 11 to 14 hours of sunlight. Hemp grown for seed is generally grown with medium to shorter varieties. Hemp varieties grown for flowers range in height with relatively wide canopies and are grown primarily to extract essential oils, the quantity of which increases when flowers are not pollinated. In the United States, because few herbicides are labeled for hemp at this time, either field layouts with sufficient spacing for open-field cultivation or cultivation under plastic mulch is preferred to combat weeds. Direct seeding into the ground is preferred for fiber and seed production at a very high plant density (0.1–0.8 million/acre) to encourage shoot growth or seed production in view of its lower economic value. However, for flowers (total delta-9 THC <0.3%), planting on raised beds is recommended at lower plant density (<10,000/acre) for high flowering without pollination because of higher economic value. Well-drained soils are preferred for hemp cultivation and require adequate moisture at planting for optimum establishment. Extended periods of flooding should be avoided. Approximately ½ to 2 inches of irrigation is recommended per week, and fertigation is a preferred method when grown on plastic-mulched beds.

1. This document is SL476, one of a series of the Department of Soil and Water Sciences, UF/IFAS Extension. Original publication date August 2020. Visit the EDIS website at <https://edis.ifas.ufl.edu> for the currently supported version of this publication.
2. Rao Mylavarapu, professor and director, UF/IFAS ANSERV Labs, Department of Soil and Water Sciences; Zachary Brym, assistant professor, agroecology; Luis Monserrate, graduate research assistant, Agronomy Department, UF/IFAS Tropical Research and Education Center; and Michael J. Mulvaney, assistant professor and cropping systems specialist, Agronomy Department, UF/IFAS West Florida REC—Milton; UF/IFAS Extension, Gainesville, FL 32611



Figure 2. Sunn hemp (*Crotalaria juncea*) grown as cover crop.
Credits: Arun Jani, graduate student, UF/IFAS Agronomy Department



Figure 3. Jute (*Corchorus olitorius*) cultivation for fiber .
Credits: Malcolm Manners, Wikimedia Commons. Licensed under the Creative Commons Attribution 2.0 Generic license.

The statewide UF/IFAS Industrial Hemp Pilot Project is researching aspects of agronomic production for hemp cultivation. While a few other state soil testing laboratories, such as those at Penn State, University of Kentucky, and the North Carolina Department of Agriculture, provide soil tests and nutrient recommendations based on research and experience, at this time no Florida-specific data on nutrient requirements and fertilization are available.

This article provides a summary of published and personal communications from different states on hemp fertilization.

Nutrient Requirements of Hemp

Currently, limited soil fertility research is available to determine accurate nutrient requirements of hemp, interpretation of soil test data, and recommendations for applications across the United States. Soil test recommendations for agronomic row crops such as corn (*Zea mays* L.) or small grains may be considered adequate in certain cases for grain or fiber hemp production as a starting point. However, research done outside the United States provides some insights on hemp nutrition. Current studies

in Canada showed that the total plant uptake was 224 lb N/acre, 53 lb P₂O₅/acre, and 236 lb K₂O/acre. Grain removal accounted for 45 lb N/acre, 21 lb P₂O₅/acre, and 11 lb K₂O/acre (Heard et al. 2007). Other studies conducted in Italy and China generally agreed with such levels of nutrient uptake (Angelini et al. 2014; Deng et al. 2019).

Guidance for Florida

Currently available information within the region and outside suggests that the data are similar to P and K recommendations for nonirrigated corn production in Florida. Research in Canada and Europe found limited response to additions of P and K for fiber and grain hemp, but likely the lack of response was due to high initial soil test levels (Vera et al. 2010; Finnan & Burke 2013; Angelini et al. 2014; Aubin et al. 2015). Recommendations for P and K nonirrigated corn in Florida (Mylavarapu et al. 2015: [SL129](#), [UF/IFAS Standardized Fertilization Recommendations for Agronomic Crops](#)) based on Mehlich-3 soil extraction are shown in Table 1.

Table 1. UF/IFAS Recommendations for P₂O₅ and K₂O for nonirrigated grain corn based on Mehlich-3 soil test levels.

Nutrient Recommendations	Soil Test Levels		
	High	Medium	Low
P ₂ O ₅ (lb/acre)	0	50	125
K ₂ O (lb/acre)	0	60	120

Because soil test correlation and calibration data are not available for Florida, the above P and K rates serve as guidance for hemp production based on the regional data until such time as Florida-specific research can be conducted.

The N applications should not exceed 150 lb N/acre, similar to the data found in the region. The N recommendation is based on research data on the crop requirement and not on a soil test. The N requirement for hemp varies with the purpose of cultivation. Seed and flower may require N rates of up to about 200 lb per acre (Struik et al. 2000; Vera et al. 2010; Angelini et al. 2014; Aubin et al. 2015; Deng et al. 2019), whereas when grown for fiber, 50 lb N/acre may be adequate, because too much N can reduce fiber quality.

N application should preferably be applied in 2–3 split applications to enhance uptake and minimize leaching in sandy soils. Typical proportion of split applications is 30 lb N/acre at planting and the rest approximately 3 weeks after planting. Continued monitoring of soil and plant tissue for N levels is important, specifically using plant tissue tests for determining sufficiency throughout the season and paying

attention to chlorosis of lower leaves. Published values on tissue nutrient levels are shown in Table 2, which may serve just as a reference point.

Table 2. Hemp leaf tissue survey ranges based on 15 mature leaves from new growth during vegetative growth in a production nursery. Data from Bryson & Mills (2014).

Macronutrients (%)		Micronutrients (ppm)	
N	3.80–4.80	Fe	100–150
P	0.19–0.25	Mn	41–93
K	1.80–2.00	B	56–105
Ca	0.40–0.60	Cu	5.0–7.1
Mg	0.15–0.30	Zn	24–52
S	0.10–0.30	Mo	0.5–1.5

Hemp is found to grow well between a soil pH range of 6.0 to 7.0. However, it was found to grow even at soil pH values above 7.0 in calcareous soils of Florida. To ensure optimum nutrient supply on acid-mineral soils of Florida, the suggested pH is 6.5.

The data provided on the UF/IFAS soil test report will consist of the following:

Extractable nutrient levels in the soil, including phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), and manganese (Mn), and guidelines for nutrient applications, as appropriate, based on the purpose of cultivation, namely fiber, seed or flowers, and based on the plant densities described previously.

Soil Testing at the UF/IFAS Extension Soil Testing Laboratory (ESTL)

Soil samples should be submitted for hemp cultivation 4–6 weeks prior to planting. The Extension Soil Testing Lab will use the specific crop code #15 for hemp sample submissions. The standard soil fertility test will provide information on soil pH, lime requirement (only when necessary), N, Mehlich-3 extractable P, K, Ca, Mg, S, Cu, Mn, and Zn. An effective nutrient management program for optimal production should couple the information obtained from soil and plant tissue analyses and be considered along with various influencing factors such as weather and soil type (Mylavarapu 2010).

Because access to Florida-specific information is not available to generate nutrient recommendations for optimal production, careful monitoring of plant and soil nutrient levels should be compared to available guidelines to estimate reasonable applications. Special consideration

should be made not to overapply fertilizer, because nutrient loading of waterbodies is a critical issue for the region.

The above guidelines will be updated as and when research data from Florida become available. The following regional data contributed towards formulation of nutrient guidelines for hemp cultivation in Florida.

Provisional Data from Regional Soil Test Labs

- Dr. Frank Sikora, University of Kentucky (personal communication, June 2020):

While research work on hemp production has been initiated at the University of Kentucky, their interim guidelines are to fertilize for P and K as in wheat (*Triticum aestivum* L.) production for Kentucky. These guidelines are similar to the guidelines found elsewhere (Ontario Ministry of Agriculture 2017; Cherney and Small 2016).

The University of Kentucky (UKY) recommends a soil pH of 6.4 for all hemp production. This pH is very close to the recommendation for most agronomic and vegetable crops grown on sandy soils and will ensure optimum nutrient solubility and availability.

Based on soil tests using the standard Mehlich-3 soil extractant procedure, UKY recommends the following:

Phosphorus: 0–120 lb P_2O_5 /acre

Potassium: 0–80 lb K_2O /acre

For nitrogen, UKY recommends:

50 lb N/acre for fiber production

100–150 lb N/acre for grain production

100–150 lb N/acre for CBD/flowers (preliminary data from field trials)

- Dr. John Spargo, Penn State (personal communication, June 2020):

Hemp is best adapted to well-drained soil with a pH between 6.0 and 7.0. Hemp does not grow well on wet soils or those with a heavy clay content. Hemp that is direct seeded is sensitive to soil crusting and soil compaction, which can occur on these soils.

Using standard Mehlich-3 extraction-based soil tests, the Agricultural Analytical Services Lab at Penn State recommends:

P: 0–120 lb P₂O₅/acre

K: 0–110 lb K₂O/acre

For nitrogen, Penn State recommends: 150 lb N/acre

- Drs. David Hardy and Michelle McGinnis, NC Department of Agriculture (personal communication, June 2020)

The target soil pH recommended for hemp production is 6.2 (for mineral soils) and 5.5 and 5.0 for mineral-organic and organic soils, respectively. Research on hemp production and nutrition has just started and so the current soil test guidelines are based on preliminary data available in the neighboring states, particularly Kentucky. The soil test lab at the NC Department of Agriculture accepts soil samples for hemp cultivation and provides the following recommendations (approximate amounts estimated using their indexing system and may vary by +/- 10 lb/acre) based on Mehlich-3 extraction method for seed production:

P: 0–90 lb P₂O₅/acre

K: 0–100 lb K₂O/acre

For Nitrogen: 100–150 lb/acre is recommended for seed production.

Preliminary results suggest that the flowering response peaks in the range of 100–120 lb N/acre. Nitrogen should not be applied in-row. For seed production, split applications are suggested on sandy soils with 50 to 75 lb N/ac at planting with the remainder applied 30 days afterward.

Table 3. Summary of current regional soil test-based hemp nutrient recommendations.

Nutrient Rates	P ₂ O ₅	K ₂ O	N	Reference*
	Recommendation rates (lb/acre)			
Penn State [#]	0–120 [#]	0–110 [#]	150	Dr. John Spargo
UKY	0–120	0–80	150	Dr. Frank Sikora
NC State	0–90	0–100	150 (120**)	Drs. David Hardy & Michelle McGinnis
Florida	0–125	0–120	150	Dr. Mylavarapu et al. (this document)

*Based on regional experience

[#] approx. values for 1250 lb/acre yield goal

^{**}based on preliminary research

The current available information and the proposed nutrient guidance for Florida is summarized in Table 3.

References

- Angelini, L. G., S. Tavarini, B. Cestone, and C. Beni. 2014. "Variation in Mineral Composition in Three Different Plant Organs of Five Fibre Hemp (*Cannabis sativa* L.) Cultivars." *Agrochimica* 58 (1): 1–18.
- Aubin, M., P. Sequin, A. Vanasse, G. Tremblay, A. F. Mustafa, and J.-B. Charron. 2015. "Industrial Hemp Response to Nitrogen, Phosphorus, and Potassium Fertilization." *Crop, Forage, and Turfgrass Management* 1 (1): 1–10. <https://doi.org/10.2134/cftm2015.0159>.
- Bryson, G. M., and H. A. Mills. 2014. *Plant Analysis Handbook IV*. Athens, GA: Micro-Macro Publishing.
- Cherney, J. H., and E. Small. 2016. "Industrial Hemp in North America: Production, Politics, and Potential." *Agronomy* 6 (4): 58. [10.3390/agronomy6040058](https://doi.org/10.3390/agronomy6040058).
- Deng, G., G. Du, Y. Yang, Y. Bao, and F. Liu. 2019. "Planting Density and Fertilization Evidently Influence the Fiber Yield of Hemp (*Cannabis sativa* L.)." *Agronomy* 9 (7): 1–10. <https://doi.org/10.3390/agronomy9070368>.
- Finnan, J., and B. Burke. 2013. "Potassium Fertilization of Hemp (*Cannabis sativa*)." *Industrial Crops & Products* 41:419–422. <https://doi.org/10.1016/j.indcrop.2012.04.055>.
- Heard, J., K. Watson, and J. Kostiuk. 2007. "Nutrient Uptake and Partitioning by Industrial Hemp." Manitoba Agronomists Conference Proceedings 2007. http://www.umanitoba.ca/faculties/afs/MAC_proceedings/proceedings/2007/Heard_poster.pdf (accessed June 14, 2020).
- Mylavarapu, R. S. 2010. "Diagnostic Nutrient Testing." *HortTech*. 20:19–22.
- Mylavarapu, R. S., D. Wright, and G. Kidder. 2015. *UF/IFAS Standardized Fertilization Recommendations for Agronomic Crops*. SL129. Gainesville: University of Florida Institute of Food and Agricultural Sciences. <https://edis.ifas.ufl.edu/ss163>.
- Ontario Ministry of Agriculture. 2016. "Growing Industrial Hemp in Ontario." <http://www.omafr.gov.on.ca/english/crops/facts/00-067.htm#fertility>.

Roth, G., J. Harper, H. Manzo, A. Collins, and L. Kime. 2018. "Industrial Hemp Production." Penn State Extension. <https://extension.psu.edu/industrial-hemp-production>.

Struik, P. C., S. Amaducci, M. J. Bullard, N. C. Stutterheim, G. Venturi, and H. T. H. Cromack. 2000. "Agronomy of Fibre Hemp (*Cannabis sativa* L.) in Europe." *Industrial Crops and Products* 11 (2–3): 107–118. [https://doi.org/10.1016/S0926-6690\(99\)00048-5](https://doi.org/10.1016/S0926-6690(99)00048-5).

Vera, C. L., S. S. Malhi, S. M. Phelps, W. E. May, and E. N. Johnson. 2010. "N, P, and S Fertilization Effects on Industrial Hemp in Saskatchewan." *Canadian Journal of Plant Science* 90 (2): 179–184. <https://doi.org/10.4141/CJPS09101>.

Root-Knot Nematode Host Status of Different Industrial Hemp Cultivars

Jacqueline D. Coburn¹ and Johan Desaeger¹

¹University of Florida, Department of Entomology and Nematology, Gulf Coast Research and Education Center, Wimauma, FL, US

Root-knot nematodes, *Meloidogyne* spp., are microscopic endoparasitic plant parasites with a wide host range. The nematode causes plant roots to form galls or knots within the roots by becoming sedentary in the vascular tissues, disrupting normal translocation of water and other nutrients, impairing plant growth and increasing susceptibility to other pathogens and pests. In Florida, because of the subtropical climate and often sandy soils, nematodes, especially root-knot (*Meloidogyne* spp.) and sting nematodes (*Belonolaimus longicaudatus*), are considered one of the main limiting factors to crop production. Florida growers are increasingly interested in new alternative crops, as many of the traditional crops in Florida, such as citrus, fruiting vegetables, and strawberries, are facing more and more pressure due to disease issues and increasing competition from abroad. With the recent removal of hemp (*Cannabis sativa*) from the controlled substances list (2018 Farm Bill and 2019 Florida Statute, SB1020), hemp is now an agricultural commodity, and interest among Florida growers is high. To support the future viability and sustainability of hemp, and considering the importance of nematodes in Florida, it is critical to assess the impact that root-knot and other nematodes may have on this crop.

A greenhouse study was set up at the Gulf Coast Research and Education Center (GCREC) of the University of Florida to evaluate the host status and susceptibility of six hemp cultivars (Helena, Tygra, Fibranova, Eletta Campana, Carmagnola, and Carmagnola Selezionata) to a mixed population of *Meloidogyne* spp. (*M. javanica* and *M. arenaria*). Cultivars were evaluated with and without nematodes in 20-cm diameter clay pots filled with steamed soil from a local field (95% sand, < 1% OM). Seeds were presoaked in distilled water for one hour, placed in a moisture chamber, and four days later germinating seeds were planted. Germination ranged from 62% to 74% with Tygra having the highest germination rate. Root-knot nematode eggs were collected from infected tomato roots from a local field using the diluted bleach method, and pots were inoculated with 10,000 root-knot nematode eggs three days after planting pregerminated seeds. Each cultivar had ten replicates—five with nematodes and five without. Cucumber (cv. Dasher II) served as a control to ensure nematode inoculum was viable. The replicates with nematodes had two plants per pot, of which one was sampled after one week and roots were stained using 12 % red food dye for evidence of nematode invasion. Height measurements were taken bi-weekly. After 60 days roots were rated for root galls (0-10 scale), root-knot eggs were extracted from roots using diluted bleach, juveniles (J2) were extracted from soil, reproduction factors ($Rf = Pf \text{ (eggs + J2s) / Pi}$) were calculated, and dry root and shoot weights were taken.

Root-knot nematode juveniles were found in all hemp cultivars after 1 week, ranging from 6 to 60 juveniles per root system, as compared to 45 juveniles in the cucumber roots. After 60 days, root-knot nematodes reproduced well on all six hemp cultivars, with roots showing small but numerous galls. Reproduction factor (Rf) was similarly high for all cultivars, ranging from 33 (Helena) to 52 (Tygra), as compared to 46 for cucumber. Plant growth (height and biomass) was not negatively affected by root-knot nematodes, but root dry weight was reduced by 44 - 52% in the cultivars Helena, Tygra, and Eletta Campana. More greenhouse and field nematode screening is planned, including testing other (root-knot) nematode species and hemp cultivars.

Development of Cannabinoids in Flowers of Industrial Hemp (*Cannabis sativa* L.): A Pilot Study

Rui Yang, Erin C. Berthold, Christopher R. McCurdy, Sarah da Silva Benevenuto, Zachary T. Brym, and Joshua H. Freeman*



Cite This: *J. Agric. Food Chem.* 2020, 68, 6058–6064



Read Online

ACCESS |



Metrics & More



Article Recommendations

ABSTRACT: A field study was performed to investigate the development of cannabinoids in flowers of industrial hemp using three day-length-sensitive and two day-length-neutral varieties. Flower samples were analyzed for cannabinoids on a weekly basis from 2 to 4 weeks postanthesis to plant senescence. Results indicate that total THC, CBD, and CBG significantly increased as flowers matured, reaching the greatest concentration during 6 to 7 weeks postanthesis. After a plateau stage of varied length for different varieties, the peak concentrations declined as plants senesced. Total THC was above the 0.3% threshold from 4 weeks postanthesis to the end of the growing season for day-length-sensitive varieties, but this only occurred during 6 to 7 weeks postanthesis for day-length-neutral varieties. The CBD/THC ratio in flowers dynamically changed during the entire reproductive stage for all of the evaluated varieties. The current study provides vital information for successful cultivation of industrial hemp.

KEYWORDS: *cannabis sativa*, THC, CBD, CBG, CBD/THC ratio

INTRODUCTION

Cannabis (*Cannabis Sativa* L.) has been domesticated and cultivated by human beings for over 4000 years as a source of food, fiber, and medicine.¹ *Cannabis* is characterized by a distinctive class of terpenophenolic compounds named cannabinoids. To date, more than 100 cannabinoids have been reported, including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG).² Although taxonomically and morphologically similar, *cannabis* can be distinguished into two unique groups, industrial hemp and marijuana, based on phytochemical profiles. *Cannabis* plants that contain a total THC concentration of $\leq 0.3\%$ on a dry weight basis are defined as “industrial hemp” by law in the United States.³ Total THC is defined by the following formula:

$$\text{concentration}_{\Delta-9-\text{THC}} + (\text{concentration}_{\Delta-9-\text{THCA}} \times 0.877)$$

Δ -9-Tetrahydrocannabinolic acid (THCA) is the molecular precursor to Δ -9-THC and is often more abundant in raw plant material. When the plant material is exposed to heat, light, or alkaline conditions, THCA will convert to Δ -9-THC through decarboxylation.² The decarboxylated forms are biologically active for medicinal or recreational use, while the acidic precursors do not share the same activity. Determining total THC content allows for the quantification of all potential Δ -9-THC. The same rule also applies to other cannabinoids, such as CBD and CBG.

Industrial hemp usually contains nonpsychoactive cannabinoids, such as CBD and CBG as major constituents. Of the few cannabinoids that have been extensively studied, CBD is primarily used for pharmaceutical and medicinal purposes. As a

vigorous antioxidative and anti-inflammatory agent, CBD may provide neuroprotection in acute and chronic cases of neurodegeneration.^{4,5} It is also reported as a promising antiepileptic agent for treatment of intractable pediatric epilepsy,⁶ with U.S. Food and Drug Administration (FDA) approval of the first drug comprised of CBD for this indication, *Epidiolex*, in 2018. Although industrial hemp is traditionally cultivated in Eurasia as a source of fiber and grain, some strains have been selected and bred for high CBD content.

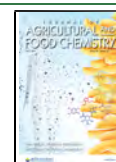
The interest in obtaining CBD from industrial hemp surged since the 2018 Farm Bill in the United States removed industrial hemp from the Controlled Substances Act (CSA) and regulated it as a “normal” crop. For this market, the value of the crop is determined by the cannabinoid content in the flowers. To maximize profit, it would be critical to harvest flowers when these compounds are at or near their maximum concentrations. Another critical factor to consider is the THC concentration. Currently, the interim final rule for industrial hemp cultivation proposed by U.S. Department of Agriculture (USDA) requires floral materials to be tested within 15 days prior to the anticipated harvest date.² All plants that exceed 0.3% total THC must be disposed of in accordance with the CSA. Information regarding development of cannabinoids in flowers of industrial hemp will be helpful for determining the date of THC regulatory testing, as well as the date of harvest

Received: February 21, 2020

Revised: May 6, 2020

Accepted: May 11, 2020

Published: May 11, 2020



for maximum profit. Unfortunately, few modern, replicated, refereed studies have explored this topic. A few investigators have tracked the evolution of cannabinoids in flowers of marijuana or in the leaves of cannabis plants cultivated in vitro and in greenhouse settings, but none of the cannabis accessions evaluated in these studies were high-CBD industrial hemp varieties that are currently cultivated in the United States.^{7–9} The objective of this study was to investigate the development of cannabinoids in the flowers of industrial hemp using high-CBD varieties under open field conditions.

MATERIALS AND METHODS

General Experimental Procedure. A field study was performed at University of Florida's North Florida Research and Education Center at Quincy, FL (30.54°N, 84.60°W) in 2019. The experimental design was a randomized complete block design with four replications. Three day-length-sensitive (DLS) varieties, including Cherry Blossom (CBL), CherryXT1 (CT1), and Cherry Wine (CW) obtained from Green Point Research (Fort Lauderdale, FL), and two day-length-neutral (DLN) varieties, including Pipeline (P) and Maverick (M) obtained from Kayagene, LLC (Salinas, CA), were evaluated. Feminized seeds were sown in the greenhouse into 128-cell seedling trays filled with PRO-MIX HP growth medium (Premier Horticulture Inc., Quakertown, PA) on June 14, 2019. Seedlings were grown under high pressure sodium light (~50 000 lm) at 16-h light and 8-h dark to maintain vegetative growth of the DLS varieties. Irrigation was supplied as needed using overhead irrigation. Uniform seedlings of each variety were transplanted to the field on July 3, 2019.

The field setup was plasticulture production with 76 cm-wide and 20 cm-high raised beds. The spacing between rows and between plants within a row was 1.8 and 1.5 m, respectively; therefore, the plant density was ~3600 plants per hectare, which is typical for current outdoor industrial hemp production in the United States.¹⁰ Anthesis, which was determined when 50% plants within a plot showed the first distinguishable pistillate flowers, occurred immediately after transplanting for the DLN varieties (July 3, 2019) and on August 7, 2019 for the DLS varieties when day length was ~13.5 h. Flower samples were taken on a weekly basis from 2 to 4 weeks postanthesis until the plants fully senesced. Flower samples (50–60 g on a fresh weight basis) were taken from the top one-third of 5 uniform plants within a plot, dried in an oven at 55 °C for 72 h, trimmed to remove stems and leaves, and ground into fine powder using a mortar and a pestle for cannabinoid analysis. In addition, 4 uniform plants within each plot were harvested for yield determination when flowers reached full maturity as indicated by the orange/brown color of pistils (August 22 and September 26, 2019 for the DLN and DLS varieties, respectively). Harvested plants were dried in a forced-air, walk-in drier at 55 °C for 72 h. After measuring the shoot biomass, flowers were trimmed by hand and flower yield was recorded. Harvest index was calculated as the ratio of flower yield to shoot biomass. Trimmed flowers were then ground into fine powder using a small coffee grinder for cannabinoid analysis.

Soils in the test site were a mixture of Tifton loamy fine sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) and Norfolk loamy fine sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) and were both well-drained. Maximum and minimum daily air temperature as well as daily rainfall was obtained from Florida Automated Weather Network (FAWN) weather station located within 2 km of the test site and illustrated in Figure 1. Total rainfall from transplanting (July 3, 2019) to harvest of the DLS varieties (September 26, 2019) was 524.5 mm. Irrigation was supplied using drip tapes under the plastic mulch based on a generalized irrigation plan for tomato plants. Irrigation rate was 6.35 mm week⁻¹ initially with an increment of 6.35 mm every 2–3 weeks until 31.75 mm week⁻¹. Total irrigation during the growing season was estimated to be ~200 mm. Fertilizer (N–P₂O₅–K₂O: 10–10–10) was applied at a rate of 112 kg N ha⁻¹ immediately prior to transplanting and disked into soils. A soluble fertilizer (N–P₂O₅–K₂O: 4–0–8) was applied

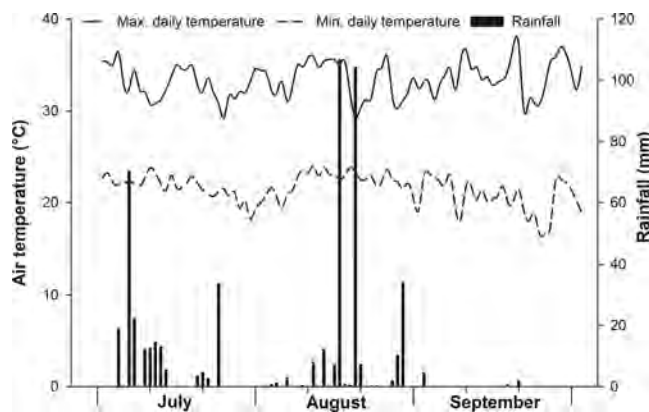


Figure 1. Maximum (max.) and minimum (min.) daily air temperature and rainfall at North Florida Research and Education Center in Quincy, FL, in 2019.

with irrigation as needed throughout the season based on an accumulated rate of 56 kg N ha⁻¹. Southern blight and corn earworm were observed in the test site, but neither was prevalent to cause severe damage.

UPLC-MS/MS Analysis of Samples. Extraction and analysis of cannabinoid was performed in a laboratory permitted under the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Industrial Hemp Pilot Project's U.S. Drug Enforcement Administration (DEA) registration to conduct chemical analysis of industrial hemp; therefore, there was not unexpected, new, and/or significant hazards or risks associated with the reported work. In brief, ground plant materials were spun with extraction solvent of methanol and water (95/5, v/v) acidified with 0.005% formic acid (plant material/solvent ratio = 1/100, w/v) using a vortex mixer for 5 min, sonicated for 5 min, and centrifuged at 3220g for 10 min at 4 °C. The supernatant was further diluted by 500-times using the same extraction solvent. Three analytical replicates were used for each sample. Commercially available calibration standards for CBD, THC, CBG, cannabidiolic acid (CBDA), THCA, and cannabigerolic acid (CBGA) as well as deuterated internal standards including delta-9-tetrahydrocannabinol-D3 (Δ -9-THC-D3) and 11-nor-9-carboxy- Δ -9-THC-D9 (11-nor-9-COOH- Δ -9-THC-D9) were obtained from Cerilliant (Round Rock, TX) and prepared using the same procedure.

Samples were then analyzed using a Waters I-Class Acquity ultraperformance liquid chromatograph equipped with a Waters Xevo TQS Micro triple-quadrupole mass spectrometer (UPLC-MS/MS, Waters Corp, Milford, MA). Positive electrospray ionization (ESI+) was used for neutral cannabinoids (e.g., CBD, CBG, and THC) while negative electrospray ionization (ESI-) was used for acidic forms (e.g., CBDA, THCA, and CBGA). The analytes were separated on an Acquity BEH C18 column (2.1 × 100 mm, 1.7 μ m, Waters Corp, Milford, MA) at a flow rate of 0.35 mL min⁻¹ using a gradient elution for 6 min. The mobile phase included water containing 0.1% formic acid (A) and methanol (B). Initially, 11% A and 89% B was held for 0.5 min, and then solvent B was linearly increased to 100% until 5.5 min followed by a sharp decrease back to the initial conditions for another 0.5 min to re-equilibrate the column. MassLynx 4.2 software (Waters Corp, Milford, MA) was used to quantify each cannabinoid using a 1/ x^2 weighing method with coefficient of determination (r^2) > 0.99 for all cannabinoids. This method has been validated in accordance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for analytical procedure validation. The recovery percentage was 98.0–114.1% for different cannabinoids at different concentrations, which was considered satisfactory. Total cannabinoid was calculated as the sum of its neutral form plus its acidic form × 0.877 and reported on a dry weight basis.

Statistical Analysis. Data were analyzed using mixed model methodology. Variety was treated as a fixed effect, while sampling date was treated as a repeated measure. Block and block × variety

interaction were random effects. To test for differences among varieties, sampling dates, and their interactions, Tukey's honestly significant difference (HSD) test was performed using SAS 9.4 software at $\alpha = 0.05$ level (SAS Institute Inc., Cary, NC). Figures were composed using SigmaPlot 14.0 software (Systat Software, San Jose, CA).

RESULTS AND DISCUSSION

Significant variety \times sampling date interaction was observed for all the evaluated cannabinoids ($P < 0.001$). Therefore, development of cannabinoids was plotted separately for each variety.

CBD. For all evaluated varieties, total CBD increased as flowers matured and reached the greatest concentration at 6 weeks postanthesis, but the trends differed afterward for different varieties (Figure 2A). For CW and the two DLN varieties, total CBD significantly dropped by 26.5, 25.1, and 17.8%, respectively, 2 weeks following the peak (8 weeks postanthesis). In contrast, a plateau of 4 and 6 weeks existed for CBL and CT1 before total CBD began to decline (Figure 2A), allowing a longer harvest window without loss of profit. Similar patterns also applied to CBDA (Figure 2C). However, the development of CBD in the DLS varieties was different from total CBD and CBDA. As shown in Figure 2B, neutral CBD initially decreased and remained relatively consistent for 5–6 weeks before it began to increase at 10 weeks postanthesis. Since the average concentration of CBDA across the whole growing season was 3- and 1.7-times greater than neutral CBD for the DLS and DLN varieties, it may be assumed that the development pattern of total CBD is primarily determined by CBDA.

The development of neutral CBD and neutral CBD/total CBD ratio synchronized (Figure 2B,D). At 6 weeks postanthesis, when total CBD peaked, neutral CBD accounted for ~17 and 40% of total CBD for the DLS and DLN varieties (Figure 2D).

THC. The development of total THC approximated total CBD. Using marijuana cultivated under greenhouse settings, De Backer et al. also found that total THC content increased strongly with plant age and reached the highest level during 5–6 weeks postanthesis.⁸ Total THC went above the 0.3% threshold at 4 weeks postanthesis and stayed above the threshold for the rest of the season for all DLS varieties (Figure 3A). Though concentration of Δ -9-THC gradually increased as flowers matured (Figure 3B), its contribution to total THC sharply dropped from >90 to <40% (Figure 3D). The development of THCA was similar to total THC except for a more evident peak at 10 weeks postanthesis (Figure 3C). Both total THC and Δ -9-THC in the DLN varieties was above the threshold at 6–7 weeks postanthesis (Figure 3A,B). Unlike total THC and Δ -9-THC, which immediately dropped following the peak, THCA in the two DLN varieties remained relatively steady until senescence (Figure 3C). In the DLN varieties, Δ -9-THC accounted for ~80% of the total THC at 6 weeks postanthesis, compared to ~31% in the DLS varieties (Figure 3D).

To maximize profit by harvesting plants when total CBD peaked at 6 weeks postanthesis, plants should be sampled and tested at 4 weeks postanthesis according to the interim final rule for industrial hemp cultivation proposed by the USDA.² At that time point, however, total THC was 0.339, 0.450, and 0.402% for CBL, CT1, and CW, respectively (Figure 3A). In such a situation, all plants may need to be disposed of and

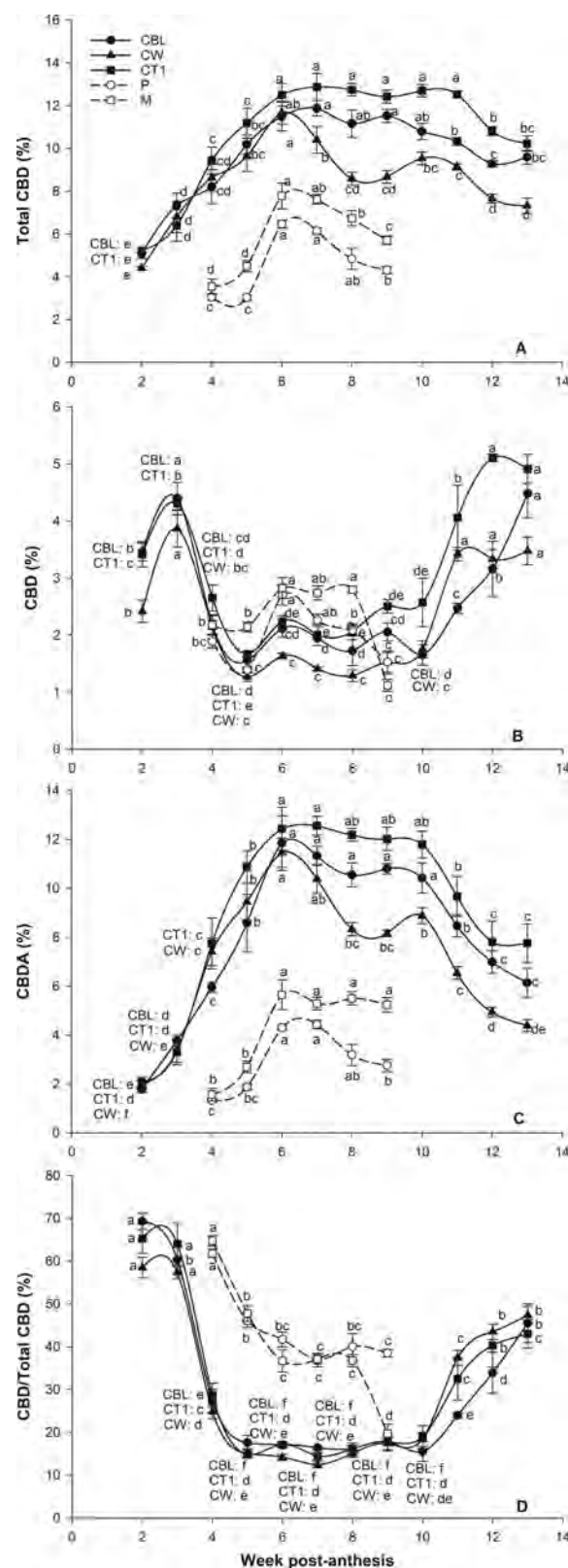


Figure 2. Development of total CBD (A), CBD (B), CBDA (C), and CBD/total CBD ratio (D) in flowers of Cherry Blossom (CBL), Cherry Wine (CW), CherryXT1 (CT1), Pipeline (P), and Maverick (M). Data represents means \pm SE ($n = 4$). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at $\alpha = 0.05$.

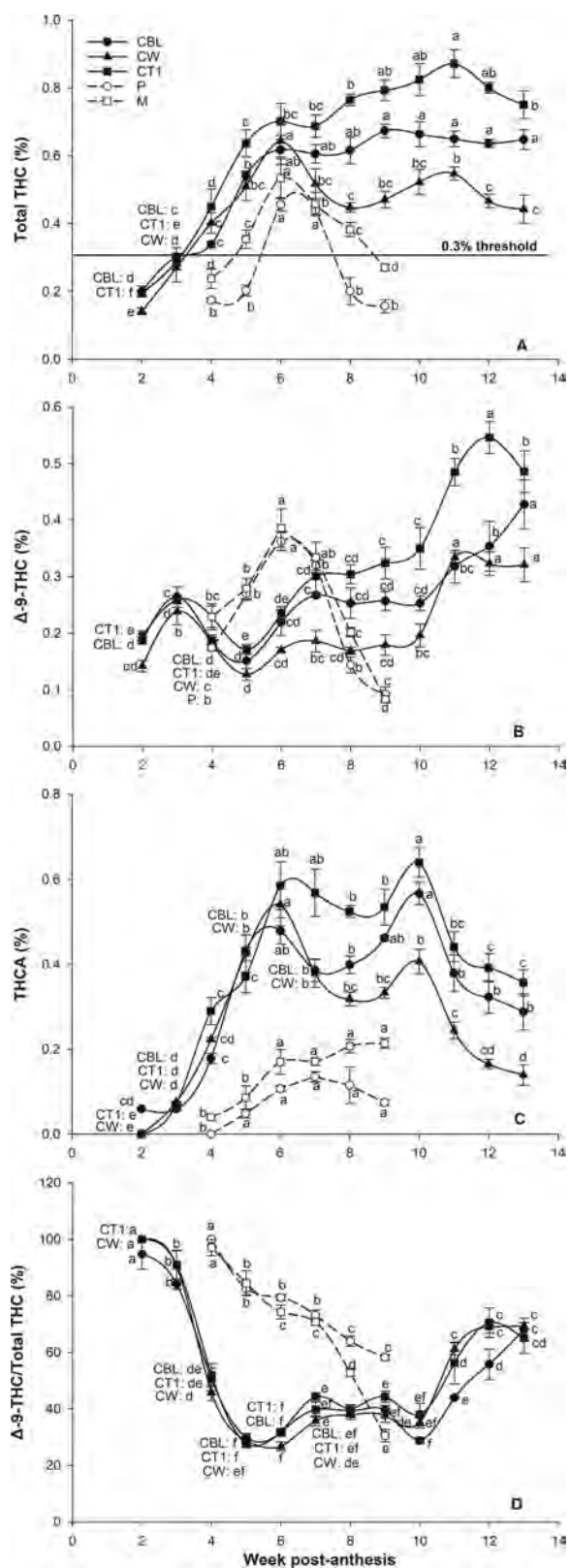


Figure 3. Development of total THC (A), Δ -9-THC (B), THCA (C), and Δ -9-THC/total THC ratio (D) in flowers of Cherry Blossom (CBL), Cherry Wine (CBL), Cherry \times T1 (CT1), Pipeline (P), and Maverick (M). Data represents means \pm SE ($n = 4$). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at $\alpha = 0.05$.

growers could lose most of the economic value of their investment. To minimize the possibility of a “false positive” test result, the USDA allows a measurement of uncertainty (i.e., “analytical error”). As long as the 0.3% threshold falls within the range of the measurement of uncertainty, the samples will be considered “having acceptable hemp THC level”. However, each state may have a different interpretation of this rule. Furthermore, sampling at 15-days prior to anticipated harvest does not guarantee a federally compliant crop at harvest as total THC concentration continued to increase from 4 to 6 weeks postanthesis (Figure 3A).

CBG. Development of total CBG was slightly different from total CBD and THC with more fluctuations (Figure 4A–C). This could be due to competition for CBGA among cannabinoids. CBGA is the precursor for both THCA and CBDA, as well as cannabichromenic acid (CBCA),¹¹ so a significant increase in THCA and CBDA during 5–6 weeks postanthesis may have resulted in the decrease in CBGA and thus neutral CBG and total CBG at 5 weeks postanthesis (Figure 4A–C). After reaching the peak at the seventh week postanthesis, total CBG significantly decreased by 43.5, 37.9, and 65.3% within 2 weeks for CBL, CT1, and CW, respectively (Figure 4A). As shown in Figure 4B,C, the development of CBGA resembled total CBG, but neutral CBG remained relatively consistent from maturity to senescence (8–13 weeks postanthesis), indicating that the CBGA synthase may be saturated. Future research on kinetics of key enzymes involved in biosynthesis of cannabinoids is necessary to better understand this topic. The evolution of neutral CBG/total CBG ratio (Figure 4D) was similar to neutral CBD/total CBD ratio (Figure 2D).

CBD/THC Ratio. For the DLS varieties, the CBD/THC ratio gradually decreased throughout the entire reproductive growing stage and followed a two-stage linear regression using combined data (Figure 4). The first stage occurred during 2–6 weeks postanthesis and the CBD/THC ratio significantly dropped from 27.7 to 18.0 ($y = -2.48x + 32.09$, $r^2 = 0.93$). During 7–13 weeks postanthesis, this ratio further declined from 19.4 to 14.9 with a less steep slope ($y = -0.77x + 24.31$, $r^2 = 0.63$). In contrast, an approximately quadratic pattern was observed for the DLN varieties. The CBD/THC ratio initially decreased by $\sim 15\%$ as flowers matured, remained relatively steady for 3 weeks (5–7 weeks postanthesis), and then significantly increased to >20 as flowers senesced (Figure 5). At 6 weeks postanthesis when both the CBD and THC peaked, the CBD/THC ratio was ~ 18 and 14 for the DLS and DLN varieties.

Previous studies reported that the CBD/THC ratio was fairly constant throughout the plant’s entire life cycle,^{7,8} which is inconsistent with results from the current study, indicating development of cannabinoids may follow different patterns for different chemotypes of cannabis and/or different hemp varieties. Cannabis can be assigned to different chemotypes based on $\log_{10}(\text{CBD}/\text{THC})$, with values <0.0 being Type I (“drug type”) and >0.0 being Type II/III (“intermediate/fiber type”).⁷ It is clear from our data that, based on this criterion, all of the varieties evaluated in the current study were Type III plants during the entire reproductive stage, indicating chemotype of the cannabis plants is fairly stable despite of the ever-changing CBD/THC values during the reproductive growth stage.

Yield. CBL tended to produce greater shoot biomass and flower yield than CW and CT1, but the harvest index was

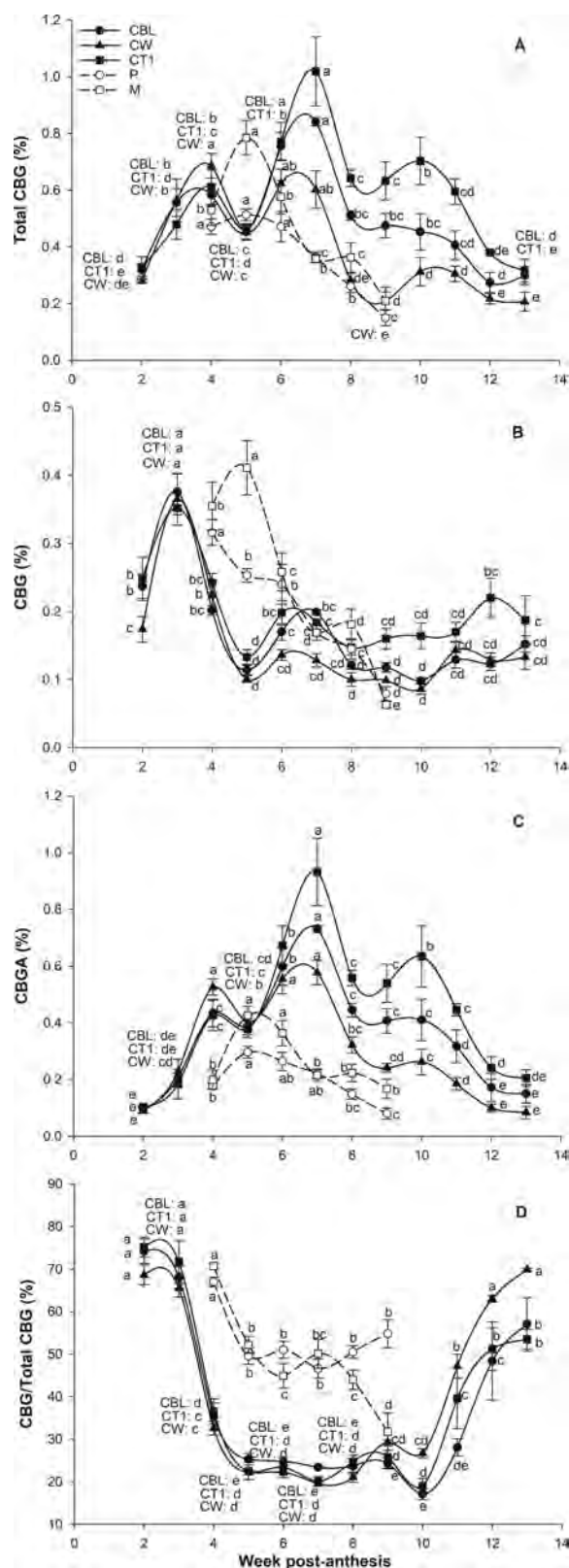


Figure 4. Development of total CBG (A), CBG (B), CBGA (C), and CBG/total CBG ratio (D) in flowers of Cherry Blossom (CBL), Cherry Wine (CW), Cherry×T1 (CT1), Pipeline (P), and Maverick (M). Data represents means ± SE ($n = 4$). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at $\alpha = 0.05$.

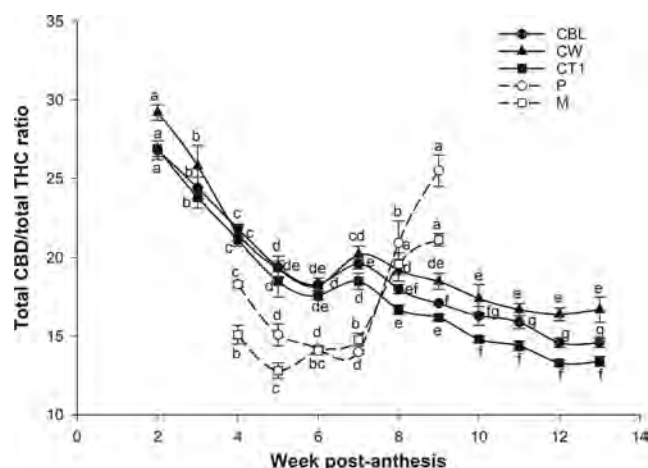


Figure 5. Development of total CBD/total THC ratio in flowers of Cherry Blossom (CBL), Cherry Wine (CW), Cherry×T1 (CT1), Pipeline (P), and Maverick (M). Data represents means ± SE ($n = 4$). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at $\alpha = 0.05$.

significantly lower (Table 1). Flower yield of $0.45 \text{ kg plant}^{-1}$ (i.e., 1 lb plant^{-1}) is generally considered optimal for DLS varieties. Flower yield for the DLS varieties in the present study was $>0.7 \text{ kg plant}^{-1}$ despite of the late planting date compared to what is typically used in other states in the southeastern United States (e.g., late May to early June).¹² Total THC, CBD, and CBG tended to be greater in CT1 relative to CBL and CW (Table 1). The two DLN varieties did not compete with the DLS varieties for flower yield or CBD content, but they showed greater harvester index than the DLS varieties. Maverick had greater yield than Pipeline, but total cannabinoid content was not significantly different (Table 1). Total THC content in all evaluated varieties except for Pipeline were above the 0.3% threshold at harvest, which was expected as they were all harvested at 7 weeks postanthesis when both total CBD and THC were at or near their maximum concentrations based on their development curves (Figure 2 and 3). Similar results were observed during the 2019 season in North Carolina, where total THC content in flowers of CBL and CW at full maturity averaged 0.52 and 0.54%.¹² However, the same varieties have tested below the THC threshold at harvest in South Carolina (Gilbert Miller, personal communication). The varieties evaluated in the current study have the required certificate of analysis (CoA) for a THC level of $\leq 0.3\%$ for parent material, but all went above threshold during reproductive growth. Without uniform testing standards, which have now been proposed by the USDA,² it is unclear when and how industrial hemp varieties were sampled and tested to obtain the CoA. There are clearly periods early in the reproductive phase of industrial hemp varieties when THC is below the critical threshold.

It is not uncommon that industrial hemp strains have tested above the THC threshold under different environmental conditions. Of the 227 high-CBD industrial hemp varieties tested in Kentucky, 141 varieties (62%), including CBL and CW are defined as “Prohibited Variety” or “Variety of Concern”, which means that they had at least one THC test result of $>0.3\%$.¹³ About 61% of the high-CBD varieties that were tested by Cornell University had total THC concentration $>0.3\%$.¹⁴ Although it has been reported that biosynthesis of cannabinoids is primarily under genetic control,^{15,16}

Table 1. Shoot Biomass, Flower Yield, and Total Cannabinoid Content in Flowers of Cherry Blossom (CBL), Cherry Wine (CWL), Cherry×T1 (CT1), Pipeline (P), and Maverick (M) at Full Maturity^a

variety ^b	shoot biomass (kg plant ⁻¹)	flower yield (kg plant ⁻¹)	harvest index ^c	total cannabinoid		
				THC (%)	CBD (%)	CBG (%)
CBL	1.13a	0.86a	0.65c	0.521ab	9.619a	0.197ab
CW	1.03b	0.74b	0.72b	0.474b	8.927a	0.189b
CT1	1.10ab	0.76ab	0.71b	0.582a	10.254a	0.260a
P	0.06d	0.02d	0.85a	0.286c	4.543c	0.200ab
M	0.12c	0.05c	0.83a	0.314c	5.562c	0.219ab

^aMeans ($n = 16$) subscribed with different lowercase letters within each column indicate significant differences among varieties at $\alpha = 0.05$. ^bDay-length-neutral varieties and day-length-sensitive were harvested on August 22 and September 26 in 2019. ^cHarvest index is calculated as the ratio of flower yield to shoot biomass.

the present study as well as results recently reported by other researchers indicates that industrial hemp accessions cultivated under different environmental conditions are expected to have different cannabinoid content. Impact of the genetic (G), environment (E), and genotype-by-environment (G×E) interaction on fiber quality of industrial hemp has been reported,¹⁷ but few studies have addressed this issue regarding cannabinoid content. Campbell et al. reported that only 1.7 and 6% of the variation in THC and CBD were explained by environment; however, irrigation was the only environmental factors evaluated in this study and fiber/grain varieties instead of high-CBD varieties were used.¹⁶ Future endeavors will be essential to better understand the stability of cannabinoid content in high-CBD varieties across different environments and thus improve the success of breeding programs.

In conclusion, cannabis production under open field conditions solely for the harvest of cannabinoids is a completely new agricultural endeavor in the United States; therefore, both growers and policy makers should be aware that environmental factors may play a role in biosynthesis of cannabinoids. Since development of cannabinoids in different hemp varieties may not follow exactly the same pattern, growers should carefully monitor content of cannabinoids postanthesis to maximize the profit and minimize the risk of above-threshold THC content. The current study indicates that the CBD/THC ratio in flowers dynamically changes during the whole reproductive growth stage. Future studies are necessary to verify these results using a larger population of hemp varieties under different environmental conditions.

AUTHOR INFORMATION

Corresponding Author

Joshua H. Freeman – North Florida Research and Education Center, University of Florida, Quincy, Florida 32351, United States; Phone: 850-875-7128; Email: joshuafr@ufl.edu

Authors

Rui Yang – Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu 610200, China; North Florida Research and Education Center, University of Florida, Quincy, Florida 32351, United States; orcid.org/0000-0003-3551-3087

Erin C. Berthold – College of Pharmacy, University of Florida, Gainesville, Florida 32610, United States; orcid.org/0000-0003-2868-8073

Christopher R. McCurdy – College of Pharmacy and Clinical and Translational Sciences Institute, Translational Drug Development Core, University of Florida, Gainesville, Florida 32610, United States

Sarah da Silva Benevenuto – North Florida Research and Education Center, University of Florida, Quincy, Florida 32351, United States

Zachary T. Brym – Tropical Research and Education Center, University of Florida, Homestead, Florida 33031, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.jafc.0c01211>

Funding

This study was part of the UF/IFAS Industrial Hemp Pilot Project sponsored by Green Roads West, LLC. Research reported in this publication was also supported by the University of Florida Clinical and Translational Science Institute, which is supported in part by the NIH National Center for Advancing Translational Sciences under Award No. UL1TR001427. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Jerry Fankhauser for providing logistics support, and Sam Maxwell, Ronnie Pugh, Aaron Reyes, and Katie Stevens for their support in sample collection and processing.

REFERENCES

- (1) Pain, S. A potted history. *Nature* **2015**, 525, S10–S11.
- (2) ElSohly, M.; Gul, W. Constituents of *cannabis sativa*. In *Handbook of Cannabis*; Pertwee, R. G., Ed.; Oxford University Press: Oxford, U.K., 2014; pp 3–22.
- (3) USDA-Agricultural Marketing Service. Establishment of a domestic hemp production program. *Fed. Reg.* **2019**, 84, 58522–58564.
- (4) Hampson, A. J.; Grimaldi, M.; Axelrod, J.; Wink, D. Cannabidiol and (–) Δ^9 tetrahydrocannabinol are neuroprotective antioxidants. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, 95, 8268–8273.
- (5) Lastres-Becker, I.; Molina-Holgado, F.; Ramos, J. A.; Mechoulam, R.; Fernandez-Ruiz, J. Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiol. Dis.* **2005**, 19, 96–107.
- (6) Devinsky, O.; Cilio, M. R.; Cross, H.; Fernandez-Ruiz, J.; French, J.; Hill, C.; Katz, R.; Di Marzo, V.; Jutras-Aswad, D.; Notcutt, W. G.; Martinez-Orgado, J.; Robson, P. J.; Rohrbach, B. G.; Thiele, E.; Whalley, B.; Friedman, D. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* **2014**, 55, 791–802.
- (7) Pacifico, D.; Miselli, F.; Carboni, A.; Moschella, A.; Mandolino, G. Time course of cannabinoid accumulation and chemotype

development during the growth of *Cannabis sativa* L. *Euphytica* **2008**, *160*, 231–240.

(8) De Backer, B.; Maebe, K.; Verstraete, A. G.; Charlier, C. Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of plants. *J. Forensic Sci.* **2012**, *57*, 918–922.

(9) Richins, R. D.; Rodriguez-Urbe, L.; Lowe, K.; Ferral, R.; O'Connell, M. A. Accumulation of bioactive metabolites in cultivated medical Cannabis. *PLoS One* **2018**, *13*, e0201119.

(10) Williams, R. A.; Williams, D. W. Cannabinoids-Human Physiology and Agronomic Principles for Production. In *Industrial hemp as a modern commodity crop*; Williams, D. W., Ed.; ASA-CSSA-SSSA: Madison, WI, 2019; p 85.

(11) Sirikantaramas, S.; Taura, F. Cannabinoids: Biosynthesis and Biotechnological Applications. In *Cannabis sativa* L. – Botany and Biotechnology; Chandra, S., Lata, H., ElSohly, M. A., Eds.; Springer: Basel, Switzerland, 2017; pp 185–186.

(12) Post, A. R.; Davis, J. M.; Bloomquist, M. G.; Learn, K. M.; Heiniger, R. W. 2019 North Carolina Hemp Strain Testing Results. *NC State Extension Publications*, 2020.

(13) Kentucky Department of Agriculture. Hemp program summary of varieties: including varieties of concern and prohibited varieties (version KDA-HEMP-2020–0117). 2020.

(14) Toth, J. A.; Stack, G. M.; Cala, A. R.; Carlson, C. H.; Wilk, R. L.; Crawford, J. L.; Viands, D. R.; Philippe, G.; Smart, C. D.; Rose, J. K.; Smart, L. B. Development and validation of genetic markers for sex and cannabinoid chemotype in *Cannabis sativa* L. *GCB Bioenergy* **2020**, *12*, 213–222.

(15) De Meijer, E. P. M.; Bagatta, M.; Carboni, A.; Crucitti, P.; Moliterni, V. M. C.; Ranalli, P.; Mandolino, G. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*. **2003**, *163*, 335–346.

(16) Campbell, B. J.; Berrada, A. F.; Hudalla, C.; Amaducci, S.; McKay, J. K. Genotype × environment interactions of industrial hemp cultivars highlight diverse responses to environmental factors. *Agrosyst. Geosci. Environ.* **2019**, *2*, 1–11.

(17) Petit, J.; Salentijn, E. M. J.; Paulo, M.-J.; Thouminot, C.; van Dinter, B. J.; Magagnini, G.; Gusovius, H.-J.; Tang, K.; Amaducci, S.; Wang, S.; Uhrlaub, B.; Mussig, J.; Trindade, L. M. Genetic variability of morphological, flowering, and biomass quality traits in hemp (*Cannabis sativa* L.). *Front. Plant Sci.* **2020**, *11*, 102.

ORIGINAL RESEARCH

Open Access



Regulatory sampling of industrial hemp plant samples (*Cannabis sativa* L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids

Erin C. Berthold¹, Rui Yang², Abhisheak Sharma^{1,3}, Shyam H. Kamble^{1,3}, Siva R. Kanumuri^{1,3}, Tamara I. King¹, Raluca Popa¹, Joshua H. Freeman², Zachary T. Brym⁴, Bonnie A. Avery^{1,3} and Christopher R. McCurdy^{1,3,5*}

Abstract

Background: In 2018, the Farm Bill mandated the United States Department of Agriculture to develop regulations governing the cultivation, processing, and marketing of industrial hemp. Industrial hemp is defined as *Cannabis sativa* L. with a total Δ -9-tetrahydrocannabinol (Δ -9-THC) content $\leq 0.3\%$. Therefore, for hemp to become an agricultural commodity, it is important to regulate production by developing standard methods for sampling and testing of the plant material.

Methods: An ultra-performance liquid chromatography-tandem mass spectrometry analytical method for the quantification of twelve cannabinoids was developed. The method was applied to a regulatory sampling trial of three hemp varieties cultivated for cannabidiol (CBD) production. Two samples were taken from 28 plants with one sample being flower only while the other was a composite sample that included flowers, leaves, and stems.

Results: The assay method was validated for specificity, range, repeatability, reproducibility, and recovery in accordance with all applicable standards for analytical methods. The results of the regulatory study indicated a significant decrease in the concentration of total Δ -9-THC and total CBD of 0.09% and 1.32%, respectively, between a flower only and a composite sample.

Conclusions: There are many factors that may influence reported total Δ -9-THC content in industrial hemp. A robust analytical method was developed to analyze hemp samples in a trial regulatory study. The results indicate that the way hemp is sampled and analyzed may influence the legality of a crop, which could have negative economic and legal consequences.

Keywords: Cannabis, Liquid chromatography–mass spectroscopy, Cannabinoid assay, Hemp, Cannabidiol, Tetrahydrocannabinol

* Correspondence: Cmccurdy@cop.ufl.edu

¹Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL, USA

³Translational Drug Development Core, Clinical and Translational Science Institute, University of Florida, Gainesville, FL, USA

Full list of author information is available at the end of the article



© The Author(s). 2020 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Introduction

Cannabis sativa L. is a source of one of the oldest known drugs in the world, cannabis, and one of the oldest known crops, industrial hemp, having been found in tombs dating back to 8000 BC (Deiana et al. 2012). The biologically active compounds in the plant are called cannabinoids, of which over one hundred have been identified to date (Hanus 2009). Being morphologically and taxonomically similar, the only characteristic that legally distinguishes industrial hemp from cannabis is the concentration of the main psychoactive component, Δ -9-tetrahydrocannabinol (Δ -9-THC), in the plant.

Regulations for sampling and testing of industrial hemp to determine total THC content are being developed. Industrial hemp was removed from the statutory definition of cannabis if the total THC content does not exceed 0.3% on a dry weight basis (Agricultural Improvement Act of 2018 2018). Total THC is defined by the following formula:

$$\text{Total THC} = \text{Concentration}_{\Delta\text{-9-THC}} + (\text{Concentration}_{\Delta\text{-9-THCA}} \times 0.877)$$

Δ -9-Tetrahydrocannabinolic acid (THCA) is the molecular precursor to Δ -9-THC. When the plant material is exposed to heat, light, or alkaline conditions, THCA will convert to Δ -9-THC. Determining total THC content allows for the quantification of all potential Δ -9-THC present in plant material.

In 2019, the University of Florida's Institute of Food and Agricultural Science (UF/IFAS) initiated cultivation studies on over 40 varieties of industrial hemp throughout the state of Florida. The first goal of this study was to develop a robust analytical method used to assess the cannabinoid content of these varieties; not only to ensure legality but also the additional ten minor cannabinoids to build a chemical fingerprint repository for each variety.

There are numerous existing methods for the detection and quantitation of cannabinoids (Gul et al. 2015, 2018; Aizpurua-Olaizola et al. 2014). Recent reviews of these methods indicated that most use either gas chromatography (GC) or liquid chromatography (LC) to separate the cannabinoids, while methods for detection include mass spectrometry, photodiode array, and ultraviolet light, among others (Nahar et al. 2020a, b; Leghissa et al. 2018; Citti et al. 2018). Here, an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed with a short run time of 6 min. At the outset of the development of this method, no others were available in the literature that was under 8 min and able to detect and quantify twelve cannabinoids. A faster method was necessary in order to analyze the samples from over forty

varieties grown across the state of Florida as part of the UF/IFAS cultivation studies. The method simultaneously separates twelve cannabinoids and quantifies them at the level of $\leq 0.05\%$ on a dry weight basis.

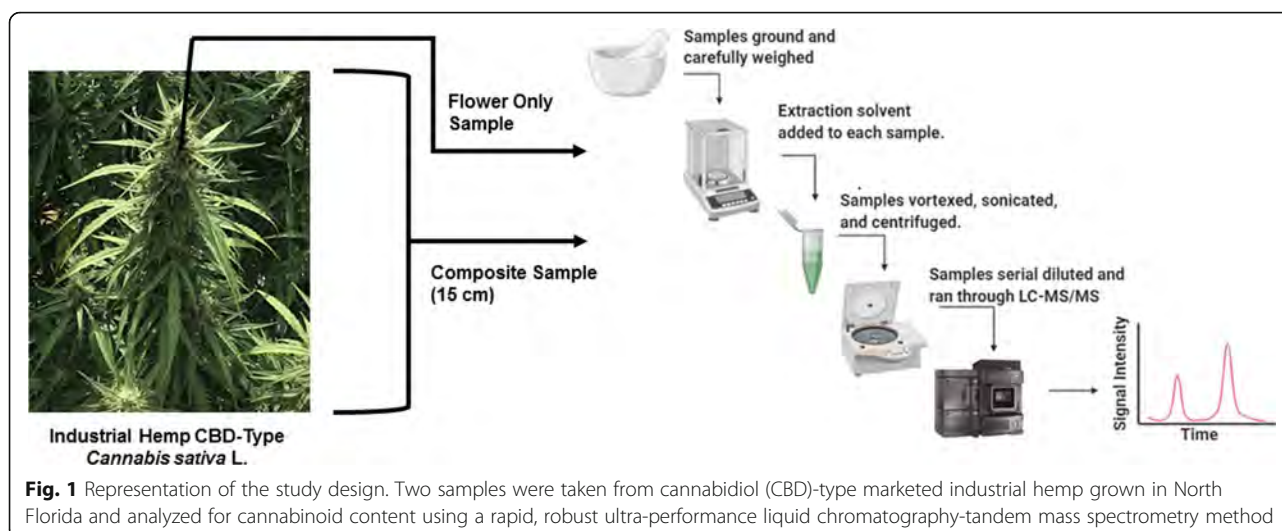
Cannabinoid concentration varies throughout the plant, with the highest concentrations in the bracts and flowers followed by significant decreases in leaves, stems, roots, and seeds (Hemphill et al. 1980; Andre et al. 2016). Currently, the Interim Final Rule for industrial hemp sampling proposed by the USDA requires inflorescent stem from the top 1/3 of the plant to be sampled, milled, and run through a screen no larger than 1.5×1.5 mm to remove larger twigs and stems (Establishment of a domestic hemp production program 2019). Alternatively, other draft sampling procedures recommend sampling the top 15–30 cm of the plant and grinding it down to uniform consistency prior to analysis (Hemp/CBD in Florida 2020; Guidance Procedures 2.0 2019). Since cannabinoid content varies throughout the plant, it is important to understand how the presence of leaves and stems in a sample for regulatory testing affects cannabinoid content. Therefore, the second goal was to investigate the cannabinoid content of a flower sample versus a 15-cm composite plant sample that included leaves and stems in three CBD-type varieties of day-length-sensitive marketed industrial hemp: cherry blossom (ChBL), cherry \times T1 (CT1), and cherry wine (CW). To the best of our knowledge, this is the first study to look specifically at regulation lengths of hemp cuttings versus floral material to investigate the potential differences in cannabinoid content. Figure 1 demonstrates the two main goals of this study and the study design.

The results of this study will provide better insight regarding the effects of plant sampling and analysis on cannabinoid content in an effort to improve industrial hemp crop production and regulatory compliance.

Materials and methods

Materials and reagents

Commercially available standards (purity $>98\%$) for cannabichromene (CBC), cannabicyclol (CBL), CBD, cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN), delta-8-tetrahydrocannabinol (Δ -8-THC), Δ -9-THC, THCA, and tetrahydrocannabivarin (THCV) were obtained from Cerilliant (Round Rock, TX, USA). Additionally, deuterated internal standards (purity $>98\%$) (IS) delta-9-tetrahydrocannabinol-D3 (Δ -9-THC-D3) and 11-nor-9-carboxy- Δ -9-THC-D9 (11-nor-9-COOH- Δ -9-THC-D9) were also obtained from Cerilliant (Round Rock, TX, USA). LC-MS grade water, methanol, and formic acid were sourced from Fisher Scientific (Fair Lawn, NJ, USA). Commercially available hops, *Humulus lupulus*, were obtained from BioKoma (Old Mill Creek, IL, USA).



Instrumentation and analytical conditions

An analytical method for quantification of cannabinoids was developed using a Waters I-Class Acquity UPLC coupled with a Waters Xevo TQ-S Micro™ triple-quadrupole mass spectrometer (MS/MS) (Milford, MA, USA). The analytes were separated on a Waters Acquity UPLC BEH C₁₈ column (2.1 × 100 mm, 1.7 μm) using a gradient elution over 6 min (Milford, MA, USA). The mobile phase was composed of water containing 0.1% formic acid (A) and methanol and acetonitrile (50:50, v/v) (B) and set at a flow rate of 0.35 mL/min. Initial conditions were 11% A and 89% B which was held for 30 s, then linearly increased to 100% B until 5.5 min, then

sharply decreased back to the initial conditions for the final 30 s to re-equilibrate the column. The weak needle wash was composed of methanol, acetonitrile, and water (1:1:2, v/v) acidified with 0.5% formic acid, while the strong needle wash was composed of methanol, acetonitrile, water, and isopropyl alcohol (1:1:1:1, v/v) acidified with 0.1% formic acid. Both wash volumes were 800 μL. The injection volume was set to 2 μL with partial needle loop overflow (to a total of 10 μL). The column oven temperature was set to 40 °C, and the autosampler temperature was set to 10 °C. Multiple reaction monitoring in positive electrospray ionization (ESI⁺) was used for neutral cannabinoids (CBC, CBL, CBD, CBDV, CBG,

Table 1 Mass spectrometer compound parameters for cannabinoids and internal standard (IS)

Cannabinoid	Mass transition (m/z)	Cone voltage (V)	Collision energy (V)
CBDV	287.1 > 165.1	6	24
THCV	287.1 > 165.1	2	22
CBN	311.2 > 223.1	2	20
CBC	315.2 > 193.1	44	18
CBD	315.2 > 193.1	30	18
Δ-8-THC	315.2 > 193.1	26	22
Δ-9-THC	315.2 > 193.1	4	18
CBL	315.2 > 235.2	28	16
CBG	317.2 > 109.0	26	32
Δ-9-THC-D3 (IS)	318.3 > 196.1	72	24
11-nor-9-COOH-Δ-9-THC-D9 (IS)	352.1 > 194.3	68	26
THCA	356.9 > 245.1	4	30
CBDA	357.1 > 107.0	4	34
CBGA	359.2 > 136.0	36	32

All transitions (m/z) were selected and compound parameters optimized for each individual cannabinoid (Δ-9-THC-D3 Δ-9-tetrahydrocannabinol-D3, 11-nor-9-COOH-Δ-9-THC-D9 11-nor-9-carboxy-Δ-9-THC-D9, CBDV Cannabidiol, CBG Cannabigerol, CBD Cannabidiol, THCV Tetrahydrocannabinol, CBN Cannabinol, Δ-9-THC Δ-9-tetrahydrocannabinol, Δ-8-THC Δ-8-tetrahydrocannabinol, CBL Cannabicyclol, CBC Cannabichromene, CBDA Cannabidiolic acid, CBGA Cannabigerolic acid, and THCA Tetrahydrocannabinolic acid) using Intellistart™ feature of MassLynx® or by manual optimization, as necessary. (V voltage, m/z mass-to-charge ratio, IS internal standard)

CBN, Δ -8-THC, Δ -9-THC, and IS Δ -9-THC-D3) while negative electrospray ionization (ESI⁻) was used for acidic cannabinoids (CBDA, CBGA, and THCA with IS 11-nor-9-COOH- Δ -9-THC-D9). The mass spectrometer settings were optimized using the IntelliStart™ feature of MassLynx® Version 4.2 (Waters, Milford, MA, USA) and transitions for each compound were selected based on which had the highest stability and abundance. The monitored transitions and instrument conditions can be seen in Table 1.

For ESI⁺, the capillary voltage was 3.0 kV, the desolvation temperature was 450 °C, the desolvation gas flow was 800 L/h, and the cone gas flow was 60 L/h. For ESI⁻, the capillary voltage was -1.75 kV, the desolvation temperature was 450 °C, the desolvation gas flow was 650 L/h, and the cone gas flow was 50 L/h. MassLynx® 4.2 software was used to acquire the data and TargetLynx™ was used to quantify the amount of each cannabinoid (Waters, Milford, MA, USA).

Preparation of calibration and quality control standards

Calibration standards (CS) were prepared from commercial stock solutions into two mix stocks of 5000 and 500 ng/mL of each cannabinoid in methanol. These mix stocks were then further diluted to provide calibration standards of 10, 50, 100, 150, 500, 1000, 1500, and 2500 ng/mL of each cannabinoid.

Quality control (QC) samples were prepared from the second set of mixed stocks to get final concentrations of 10, 75, 750, and 1750 ng/mL. Sample preparation of QCs used the same conditions as plant samples, which included vortex mixing, sonication, and centrifugation prior to analysis.

An IS stock was made at 500 ng/mL and added to CS, QC, and test samples to get a final concentration of 50 ng/mL.

Stock stability was assessed on the mix stock solutions after 6 months of storage at -20 °C. The mixed stock was used to prepare a standard curve while fresh QC samples of each individual cannabinoid were generated and quantified against the mixed stock curve.

Sample preparation

Plant samples were dried in an oven at 55 °C for 72 h to ensure plant material was brittle. This time and temperature were chosen to minimize decarboxylation (Wang et al. 2016; Iffland et al. 2016). Samples were ground into a fine powder using a small coffee grinder. One of the two samples from the same plot was ground as the whole inflorescence with the stem and leaf included (top 15 cm) to obtain a composite sample, whereas the other one was trimmed, and only flowers were ground. For composite samples, the stem and

leaves on average accounted for $9.4 \pm 2.8\%$ of the dried weight of the sample.

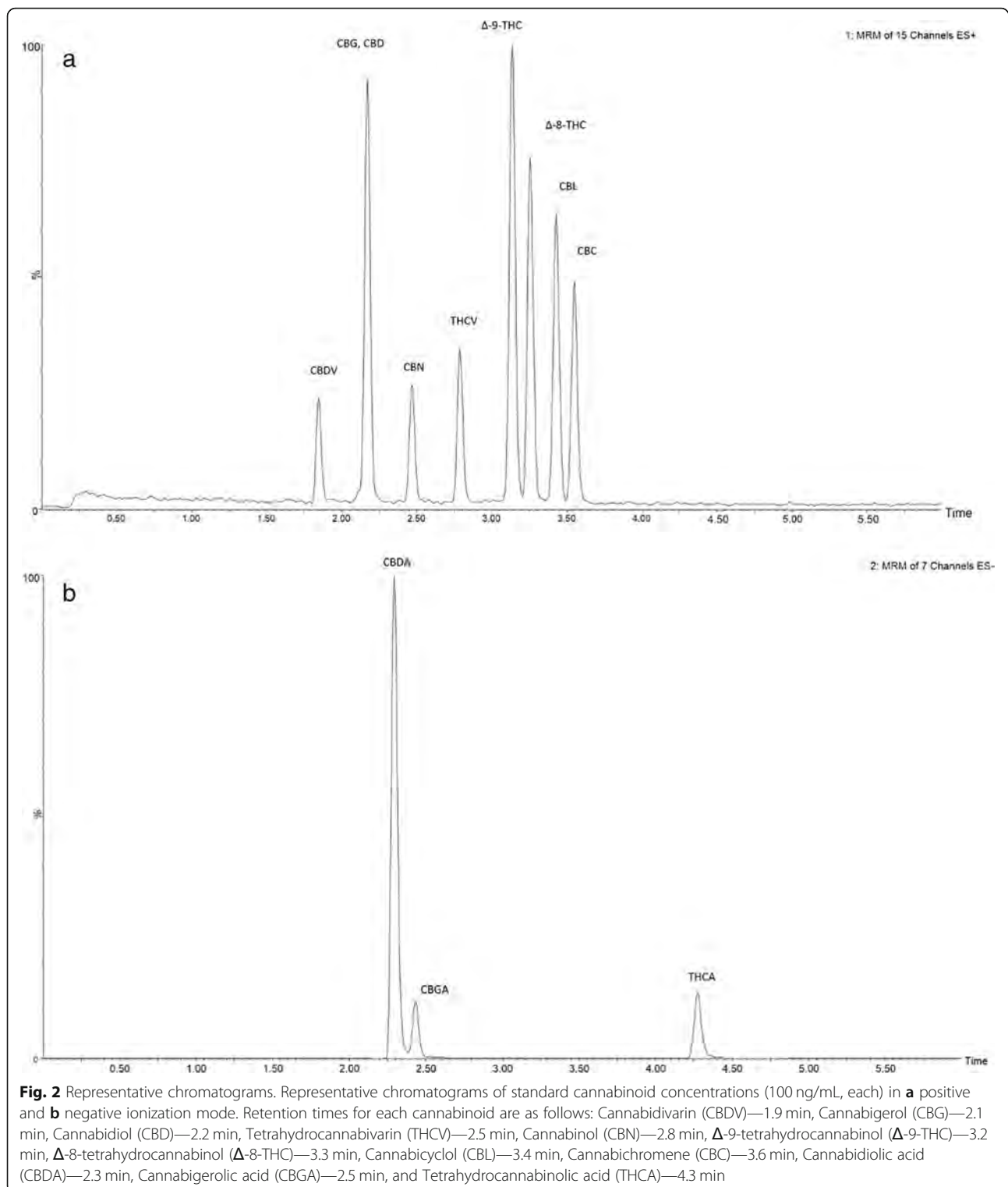
The dried, ground industrial hemp plant samples were carefully weighed in triplicate and cannabinoids were extracted by adding a solution of methanol and water (95:5, v/v) acidified with 0.005% formic acid. The plant material to solvent concentration ratio was 1:100 (w/v). After the addition of the extraction solvent, samples were vortex mixed for 5 min, sonicated for 5 min, and centrifuged at 4 °C, 3220×g for 10 min. Once spun down, the supernatant was serially diluted using a fresh extraction solvent to an appropriate final sample concentration to fall within the quantification range and meet range requirements.

Analytical method validation

The method was validated for specificity, range, repeatability, reproducibility, and recovery in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2(R1) Guidelines for analytical procedure validation (Validation of Analytical Procedures: Text and Methodology 2001). In addition, the Association of Official Analytical Chemists Standard Method Performance Requirements (AOAC SMPR) 2019.003 for quantification of cannabinoids in low THC varieties of hemp plant material was also followed (Standard Method Performance Requirements (SMPRs) for Quantification of cannabinoids in plant materials of hemp (Low THC Varieties Cannabis sp.) 2019).

Application to mock regulatory study

The study was performed at the University of Florida's North Florida Research and Education Center (NFREC) at Quincy, FL (30.54°N, 84.60°W) in 2019. The experimental design was a randomized complete block design with 4 replications. The seeds of ChBL, CT1, and CW were sown in the greenhouse into 128-cell seedling trays filled with PRO-MIX HP growth medium (Premier Horticulture Inc., Quakertown, PA, USA) on June 14, 2019. Seedlings were grown under supplemental lighting (16-h light and 8-h dark) to maintain vegetative growth. Irrigation was supplied as needed using overhead irrigation. Uniform seedlings of each variety were transplanted to the field on July 3, 2019. The field was set up with 20-cm high raised beds covered with plastic. Irrigation was supplied daily using drip tapes. Fertilizer (N-P₂O₅-K₂O: 10-10-10) was applied at a rate of 112 kg ha⁻¹ immediately prior to transplanting and disked into soils. A soluble fertilizer (N-P₂O₅-K₂O: 4-0-8) was applied with irrigation as needed throughout the season based on an accumulated rate of 56 kg N ha⁻¹. Anthesis was observed on August 7, 2019, when the day-length was ~13.5 h. Two top 15 cm samples were taken on October 10, 2019 from 26 experimental plots resulting in 56 samples.



Statistical analysis

R Studio version 3.6.0 was used for statistical analysis (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed paired *t* test was performed for each cannabinoid to analyze if a difference existed between the

sampling method (flower vs composite) at a significance level of $\alpha \leq 0.05$. A two-way ANOVA was performed to determine the effect of variety and treatment on cannabinoid levels and if there existed any interaction between the factors: sample type and variety. Additionally, the

Table 2 Stock solution stability results for each cannabinoid

Concentration (ng/mL)	Cannabinoid					
	CBC % Nominal ± SD	CBD % Nominal ± SD	CBDA % Nominal ± SD	CBDV % Nominal ± SD	CBG % Nominal ± SD	CBGA % Nominal ± SD
75	98.2 ± 11.9	94.1 ± 1.3	99.4 ± 1.5	93.7 ± 3.0	96.7 ± 6.0	96.9 ± 3.0
1750	102.8 ± 4.5	109.3 ± 2.3	99.9 ± 13.8	100.3 ± 7.9	91.6 ± 0.7	103.1 ± 0.3
Concentration (ng/mL)	Cannabinoid					
	CBL % Nominal ± SD	CBN % Nominal ± SD	Δ-8-THC % Nominal ± SD	Δ-9-THC % Nominal ± SD	THCA % Nominal ± SD	THCV % Nominal ± SD
75	108.4 ± 4.3	108.1 ± 5.9	98.6 ± 6.0	92.0 ± 1.9	95.1 ± 8.2	93.5 ± 4.4
1750	101.2 ± 5.3	108.2 ± 0.4	99.0 ± 2.9	97.8 ± 1.1	94.8 ± 7.9	93.0 ± 3.3

Mixed stocks were stored at −20 °C for 6 months. Individual stocks were freshly prepared and % nominal was calculated as observed concentration/nominal concentration *100 from the mixed stock generated curve. All values represent as mean ± standard deviation ($N = 3$)

CBDV Cannabidiol, CBG Cannabigerol, CBD Cannabidiol, THCV Tetrahydrocannabinol, CBN Cannabinol, Δ-9-THC Δ-9-tetrahydrocannabinol, Δ-8-THC Δ-8-tetrahydrocannabinol, CBL Cannabicyclol, CBC Cannabichromene, CBDA Cannabidiolic acid, CBGA Cannabigerolic acid, and THCA Tetrahydrocannabinolic acid

agreement between sets was evaluated by calculating the intraclass correlation coefficient. For CBD, CBG, and Δ-9-THC, the neutral and acidic forms were added together using the following formula to obtain the total cannabinoid content to be used in statistical analyses:

$$\text{Total content} = \text{Concentration}_{\text{neutral}} + (\text{Concentration}_{\text{acid}} * 0.877)$$

Results

UPLC-MS/MS method development and validation

A rapid and reliable method was developed for the quantification of 12 cannabinoids in hemp samples. Representative chromatograms for both positive and negative ionization modes at 100 ng/mL are shown in Fig. 2.

Specificity

The method was validated for specificity by separating four compounds with the same molecular weight (CBC, CBD, Δ-8-THC, Δ-9-THC, CBL) which can be seen baseline separated in Fig. 2.

Calibration range, linearity, and stock stability

The recommended ranges according to the AOAC SMPR 2019.003 are 0.05 to 5% w/w for all cannabinoids except CBD and CBDA, which have a recommended range of 0.05 to 35% w/w.

Based on the recommendations, a calibration range of 10–2500 ng/mL representing 0.05–35% w/w of cannabinoid content was selected. Linearity was seen over this range using a $1/x^2$ weighing method resulting in a correlation coefficient >0.99 for all cannabinoids. The concentration of 10 ng/mL was chosen as the limit of quantification for all cannabinoids as it always resulted in a signal to noise ratio of greater than 10:1. The limit of detection for this method was determined to be 1 ng/mL as it always resulted in a signal to noise ratio greater than 3:1 for all cannabinoids.

Freshly prepared QC samples were made for each individual alkaloid (75 and 1750 ng/mL, $N = 3$). These were then quantified using a curve generated from a mixed stock solution that had been stored at −20 °C for 6 months. Accuracy of the individual cannabinoids fell within 15% of the nominal concentration (85–115) at LQC and HQC when quantified against the mixed stock calibration curve. This indicates that cannabinoids do not degrade in mixed stock within 6 months. The results can be seen in Table 2.

Repeatability, reproducibility, and recovery

Over a period of 3 days, six replicates at four concentrations (10, 75, 750, and 1750 ng/mL) were analyzed to determine the repeatability (intra-day) and reproducibility (inter-day) of the method. The accuracy and precision for intra- and inter-day samples for each individual cannabinoid can be seen in Tables 3 and 4. Precision was measured as the percent relative standard deviation which was calculated by multiplying the standard deviation by 100 then dividing this value by the mean. Accuracy was measured as percent bias which was calculated by subtracting the observed mean from the nominal concentration then dividing this value by the nominal concentration prior to multiplying by 100 to get the percent bias. For repeatability, the percent relative standard deviation values were always ≤5% at 0.05% w/w, ≤3% in the 0.05–5% w/w range, and ≤2% for the 5–35% w/w range.

For reproducibility, the relative standard deviation fell within ≤10% at 0.05% w/w, ≤8% in the 0.05–5% w/w range, and ≤6% for the 5–35% w/w range.

Recovery was measured by spiking dried *Humulus lupulus* plant samples, used because they come from the same taxonomical family as cannabis, Cannabaceae, with a known quantity of cannabinoids. These samples were then prepared in the exact same way as an analytical sample and ran through the UPLC-MS/MS method to

Table 3 Intra-day accuracy and precision for cannabinoids of the assay method. The results verify the repeatability of the assay method as required by AOAC SMRP 2019.003 (Standard Method Performance Requirements (SMPRs) for Quantification of cannabinoids in plant materials of hemp (Low THC Varieties Cannabis sp.) 2019)

Concentration (ng/mL)	Cannabinoid			Cannabinoid			Cannabinoid		
	CBC			CBD			CBDA		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% bias)
10	10.3 ± 0.5	4.6	2.7	10.3 ± 0.5	4.9	3.2	10.5 ± 0.3	3.3	4.8
75	72.3 ± 0.9	1.3	−3.7	72.3 ± 1.4	1.9	−3.6	74.2 ± 2.0	2.7	−1.1
750	768.6 ± 18.7	2.4	2.5	755.6 ± 17.6	2.3	0.7	739.6 ± 20.5	2.8	−1.4
1750	1856.8 ± 31.8	1.7	6.1	1842.8 ± 30.2	1.6	5.3	1760.9 ± 33.8	1.9	0.6
Concentration (ng/mL)	Cannabinoid			Cannabinoid			Cannabinoid		
	CBDV			CBG			CBGA		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	9.2 ± 0.2	2.2	−8.4	11.0 ± 0.4	3.4	10.5	10.5 ± 0.5	4.9	5.1
75	73.2 ± 1.4	1.9	−2.3	69.0 ± 2.2	3.2	−8.1	72.1 ± 0.6	0.9	−3.9
750	767.8 ± 18.6	2.4	2.4	760.2 ± 17.2	2.3	1.4	732.7 ± 15.9	2.2	−2.3
1750	1826.8 ± 26.8	1.5	4.4	1851.1 ± 29.3	1.6	5.8	1754.8 ± 48.6	2.8	0.3
Concentration (ng/mL)	Cannabinoid			Cannabinoid			Cannabinoid		
	CBL			CBN			Δ-8-THC		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	10.3 ± 0.4	3.5	2.6	10.1 ± 0.5	4.6	1.3	10.6 ± 0.4	3.6	5.6
75	76.4 ± 1.4	1.8	1.9	79.1 ± 2.2	2.8	5.4	79.2 ± 1.7	2.1	5.6
750	786.9 ± 16.8	2.1	4.9	757.0 ± 18.1	2.4	0.9	792.9 ± 16.4	2.1	5.7
1750	1833.8 ± 25.0	1.4	4.8	1732.9 ± 13.7	0.8	−1.0	1784.6 ± 23.9	1.3	2.0
Concentration (ng/mL)	Cannabinoid			Cannabinoid			Cannabinoid		
	Δ-9-THC			THCA			THCV		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	9.8 ± 0.3	2.9	−1.5	10.3 ± 0.5	4.0	3.4	9.9 ± 0.4	5.5	−0.9
75	79.7 ± 1.7	1.9	6.3	77.6 ± 1.6	2.1	3.5	79.9 ± 1.8	2.3	6.6
750	753.8 ± 16.4	1.3	0.5	710.8 ± 15.5	2.2	−5.2	771.8 ± 14.8	1.9	2.9
1750	1760.7 ± 23.9	0.8	0.6	1625.1 ± 24.0	1.5	−7.1	1763.4 ± 25.6	1.5	0.8

Precision was measured as the percent relative standard deviation which was calculated by multiplying the standard deviation by 100 then dividing this value by the mean. Accuracy was measured as percent bias which was calculated by subtracting the observed mean from the nominal concentration then dividing this value by the nominal concentration prior to multiplying by 100 to get the percent bias

SD Standard deviation, %RSD Percent relative standard deviation, CBDV Cannabidivarin, CBG Cannabigerol, CBD Cannabidiol, THCV Tetrahydrocannabivarin, CBN Cannabinol, Δ-9-THC Δ-9-tetrahydrocannabinol, Δ-8-THC Δ-8-tetrahydrocannabinol, CBL Cannabicyclol, CBC Cannabichromene, CBDA Cannabidiolic acid, CBGA Cannabigerolic acid, and THCA Tetrahydrocannabinolic acid

determine recovery percentage. Recovery was calculated by dividing the observed concentration by the nominal concentration and multiplying this value by 100. The recovery percentages are shown in Table 5 and all were within the ranges recommended by AOAC SMRP 2019.003.

Uncertainty

The uncertainty for each cannabinoid at each concentration level can be calculated using the formula $U = k * RSD$ provided by the FDA Office of Regulatory Affairs (ORA Laboratory Manual 2019). The relative standard deviation used in this calculation was the one generated from the

Table 4 Inter-day accuracy and precision for cannabinoids of the assay method. The results verify the reproducibility of the assay method as required by AOAC SMRP 2019.003 (Standard Method Performance Requirements (SMPRs) for Quantification of cannabinoids in plant materials of hemp (Low THC Varieties Cannabis sp.) 2019)

Concentration (ng/mL)	Cannabinoide			CBD			CBDA		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	10.3 ± 0.4	4.1	3.2	10.2 ± 0.4	4.0	1.8	10.4 ± 0.6	5.3	4.1
75	72.5 ± 4.6	6.4	−3.3	72.5 ± 2.4	3.3	−3.3	76.3 ± 4.7	6.1	1.7
750	760.1 ± 28.1	3.7	1.3	749.9 ± 21.4	2.9	0.0	739.0 ± 30.5	4.1	−1.5
1750	1868.4 ± 58.4	3.1	6.8	1841.3 ± 48.9	2.7	5.2	1734.9 ± 76.7	4.4	−0.9
Concentration (ng/mL)	CBDV			CBG			CBGA		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	9.6 ± 0.6	6.0	−3.8	10.3 ± 0.8	8.1	2.7	10.6 ± 0.9	8.2	5.7
75	73.6 ± 3.6	4.8	−1.8	70.5 ± 3.7	5.3	−6.0	74.2 ± 4.2	5.7	−1.0
750	765.8 ± 27.8	3.6	2.1	755.6 ± 24.9	3.3	0.7	735.0 ± 19.8	2.7	−2.0
1750	1852.4 ± 56.3	3.0	5.9	1880.0 ± 58.9	3.1	7.4	1731.0 ± 77.0	4.4	−1.1
Concentration (ng/mL)	CBL			CBN			Δ-8-THC		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	10.3 ± 0.5	4.4	3.0	9.8 ± 0.4	4.3	−1.8	10.3 ± 0.4	4.3	3.3
75	74.5 ± 4.6	6.2	−0.7	76.8 ± 4.0	5.1	2.4	75.4 ± 5.0	6.7	0.5
750	779.6 ± 25.6	3.3	4.0	755.9 ± 29.2	3.9	0.8	778.8 ± 35.5	4.6	3.8
1750	1877.3 ± 67.3	3.6	7.3	1736.0 ± 55.0	3.2	−0.8	1762.2 ± 52.5	3.0	0.7
Concentration (ng/mL)	Δ-9-THC			THCA			THCV		
	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)	Measured Concentration (mean ± SD)	Precision (%RSD)	Accuracy (% Bias)
10	9.8 ± 0.6	5.9	−1.6	9.8 ± 0.7	6.8	−1.9	10.0 ± 0.4	4.4	0.0
75	77.9 ± 3.3	4.2	3.8	79.0 ± 4.1	5.2	5.3	78.3 ± 3.4	4.3	4.3
750	758.2 ± 24.8	3.3	1.1	719.7 ± 34.1	4.7	−4.0	760.4 ± 33.1	4.4	1.4
1750	1741.8 ± 64.9	3.7	−0.5	1621.6 ± 100.2	6.2	−7.3	1738.6 ± 59.1	3.4	−0.6

Precision was measured as the percent relative standard deviation which was calculated by multiplying the standard deviation by 100 then dividing this value by the mean. Accuracy was measured as percent bias which was calculated by subtracting the observed mean from the nominal concentration then dividing this value by the nominal concentration prior to multiplying by 100 to get the percent bias

SD Standard deviation, %RSD Percent relative standard deviation, CBDV Cannabidivarin, CBG Cannabigerol, CBD Cannabidiol, THCV Tetrahydrocannabivarin, CBN Cannabinol, Δ-9-THC Δ-9-tetrahydrocannabinol, Δ-8-THC Δ-8-tetrahydrocannabinol, CBL Cannabicyclol, CBC Cannabichromene, CBDA Cannabidiolic acid, CBGA Cannabigerolic acid, and THCA Tetrahydrocannabinolic acid

inter-day validation. The coverage factor at 95%, k , for $N = 18$ would be 2.11. Therefore, for Δ-9-THC near the threshold for legality, the uncertainty is 8.86% or ±0.03. When reporting values for regulatory purposes, the concentration of the cannabinoid is presented with the uncertainty limit added as the standard deviation.

Study results

The full cannabinoid profile was obtained for 56 plant samples. The major cannabinoids present in all samples were CBC, CBD, CBDA, CBG, CBGA, Δ-9-THC, and THCA. All other cannabinoids were below the limit of quantification (≤0.05% w/w).

Table 5 Percent recovery study results recovery was calculated as Observed Concentration/Nominal Concentration *100. Data represented as mean \pm standard deviation (SD)

Concentration (ng/ mL)	Cannabinoid					
	CBC	CBD	CBDA	CBDV	CBG	CBGA
	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD
10	115.0 \pm 0.8	100.6 \pm 1.5	112.4 \pm 2.1	117.9 \pm 1.2	114.1 \pm 2.3	98.4 \pm 1.5
75	108.6 \pm 0.5	106.4 \pm 0.2	109.7 \pm 3.5	106.9 \pm 0.1	106.2 \pm 1.9	101.2 \pm 4.3
750	103.3 \pm 2.2	102.3 \pm 2.4	100.5 \pm 1.6	101.5 \pm 2.6	101.6 \pm 1.7	98.0 \pm 0.3
1750	106.3 \pm 1.5	103.2 \pm 1.7	100.3 \pm 4.0	104.3 \pm 1.7	105.1 \pm 1.0	102.8 \pm 1.1
Concentration (ng/ mL)	Cannabinoid					
	CBL	CBN	Δ -8-THC	Δ -9-THC	THCA	THCV
	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD	Mean % Recovery \pm SD
10	110.1 \pm 1.7	104.6 \pm 1.7	106.3 \pm 3.4	107.1 \pm 2.9	108.0 \pm 3.4	105.1 \pm 1.8
75	108.8 \pm 0.6	110.6 \pm 0.1	110.5 \pm 1.8	107.3 \pm 1.2	110.1 \pm 1.5	108.6 \pm 1.1
750	103.7 \pm 1.6	101.9 \pm 1.9	106.0 \pm 1.6	101.1 \pm 1.4	98.5 \pm 1.4	101.2 \pm 1.3
1750	104.5 \pm 1.5	100.7 \pm 1.7	104.5 \pm 1.9	101.1 \pm 0.2	100.2 \pm 1.8	101.3 \pm 1.8

CBDV Cannabidiol, CBG Cannabigerol, CBD Cannabidiol, THCV Tetrahydrocannabivarin, CBN Cannabinol, Δ -9-THC Δ -9-tetrahydrocannabinol, Δ -8-THC Δ -8-tetrahydrocannabinol, CBL Cannabicyclol, CBC Cannabichromene, CBDA Cannabidiolic acid, CBGA Cannabigerolic acid, and THCA Tetrahydrocannabinolic acid

A two-tailed paired *t* test for total CBG and CBC gave a result of no significant difference between flower and composite samples. Alternatively, the results of the paired *t* test for total THC and total CBD indicated a significant difference of 0.09 and 1.32% between flower and composite samples, respectively. The intraclass correlation coefficient for each set of tests was also calculated. For total THC and total CBD, there was a poor agreement between the sets while total CBG had a moderate agreement and CBC had a good agreement between tests, providing further assurance that the measured difference in groups was valid (Table 6).

Further, the individual varieties were examined to investigate if variation existed between the variety for the sample type. A two-way ANOVA was performed investigating the combined effect of sample type and variety. For both

total CBD and total THC, there was no significant difference in the means of the interaction of the two factors, with *p* values of 0.31 and 0.38, respectively.

Discussion

The method developed was validated for the analysis of industrial hemp samples and determined to be rapid, reliable, and robust. The method had a short run time of 6 minutes which did not allow for CBD and CBG to be separated chromatographically but this was simply solved with mass detection of unique fragmentation patterns attributable to each cannabinoid. Cannabinoid assay methods available in the literature to simultaneously quantify over ten cannabinoids were 8 minutes or more, so the short run time of the developed method will greatly improve throughput for laboratories analyzing hemp for regulatory purposes.

Table 6 Statistical analysis of CBC, total CBD, total CBG, and total THC in flower versus composite samples

Cannabinoid	Sample type		Mean difference	Intraclass correlation coefficient
	Flower (% w/w)	Composite (% w/w)		
CBC	0.27 \pm 0.19	0.25 \pm 0.19	0.02	0.79
Total CBD	12.3 \pm 2.51	11.0 \pm 1.98	1.32*	0.32
Total CBG	0.28 \pm 0.11	0.25 \pm 0.11	0.03	0.55
Total THC	0.69 \pm 0.16	0.60 \pm 0.13	0.09*	0.29

Values represent the mean \pm standard deviation. A two-tailed paired *t* test was performed to determine if there was a significant mean difference between the composite and flower only samples. *indicates a significant difference at $p \leq 0.05$. Intraclass correlation coefficient was also calculated for each group to determine the degree to which values from the same group agree. This coefficient is interpreted as follows: <0.5 poor agreement, 0.5–0.75 moderate agreement, 0.75–0.9 good agreement, and >0.9 excellent agreement

CBC Cannabichromene, CBD Cannabidiol, CBG Cannabigerol, THC Tetrahydrocannabinol

As an LC method, the acidic and neutral cannabinoids are quantified individually and total THC must be calculated after analysis. For this project, that was important in order to be able to investigate and define the concentration of acidic and neutral compounds separately in the plant over time (Yang et al. 2020). But in the regulatory setting, where only total THC needs to be reported, GC methods will calculate this value in the detector because as the sample is heated the acidic compounds are converted to their neutral form.

When considering detection methods, MS is sensitive and selective which is ideal when monitoring many compounds that are similar in structure and mass, as is the case with phytocannabinoids in hemp (Nie et al. 2019), but thus requires samples to be diluted extensively prior to analysis. This method can detect cannabinoids at a level of 0.005% on a dry weight basis. Other detection systems, such as UV or DAD are not as sensitive and selective but allow for higher concentrations of analytes to be injected for analysis which may decrease sample preparation time (Wang et al. 2016; Vaclavik et al. 2019; Zivovinic et al. 2018).

Other countries have designated standard equipment and methods for the determination of total THC in industrial hemp (Industrial Hemp Technical Manual 2004). As it stands, the United States has not selected a standard method but studies have already indicated that cannabinoid test results are inconsistent between laboratories (Jikomes and Zoorob 2018). Therefore, it is imperative that standard methods be suggested to decrease the potential for variation between results.

In addition to the variability that may exist between laboratories and testing methods, there is also the potential for variability when considering how plants are sampled. The results of this study show that there is the potential for significant differences in cannabinoid content based on which plant part is sampled. A decrease in 0.09% w/w of total THC was seen between a flower sample and a 15-cm composite sample. As the margin for error when it comes to a crop being legal or illegal at the federal level is very slim, these results are important to consider when drafting sampling guidelines for industrial hemp crops. If the process of sampling is not standardized, the same crop could test above or below the legal threshold based on the manner in which the crop was sampled. In this study, only one length (15 cm) was investigated, so future studies would consider various lengths to see how to dilute a flower sample becomes as more leaf and stem biomass is added. Environmental factors such as soil quality, geographical location, temperature, and rainfall, among others, could also be influential in the development of cannabinoids so only sampling 26 plants grown in the same area is insufficient. Further studies could examine plants grown in various regions to determine if the difference between

flower only and composite samples prevails. Also, this study only examined three CBD-type hemp varieties, but in the future, this research could be expanded to include fiber, grain, and dual-purpose industrial hemp varieties.

Though sample analysis and sample type differences may seem insignificant when considered individually, when combined, there is the possibility of significant legal and economic ramifications.

Conclusions

From a regulatory perspective, these results indicate that the way industrial hemp samples are taken and analyzed may influence the legality of a crop. To determine the relative difference, the percent change was calculated using the formula:

$$\text{Percent Change} = (\text{Mean difference} / \text{Overall mean}) * 100$$

For total CBD, the percent change was 11% and for total THC the percent change was 14% between sampling types. When this is added to the uncertainty of the method, which was calculated to be 9%, there is an opportunity for a 23% difference in total THC. This has the potential to influence whether crop tests as industrial hemp or cannabis. As any industrial hemp crop testing over the legal limit must be destroyed, the consequences of having a significant deal of variation in sampling and analysis are substantial. When considering the many factors involved that could influence the testing results for industrial hemp and with the threshold for legality being so low, descriptive and strict sampling and testing methods must be defined in order to standardize and achieve consistent results.

Abbreviations

11-nor-9-COOH- Δ -9-THC-D9: 11-nor-9-carboxy- Δ -9-THC-D9; Δ -8-THC: Delta-8-tetrahydrocannabinol; Δ -9-THC: Delta-9-tetrahydrocannabinol; Δ -9-THC-D3: Delta-9-tetrahydrocannabinol-D3; AOAC SMPR: Association of Official Analytical Chemists Standard Method Performance Requirements; CBC: Cannabichromene; CBD: Cannabidiol; CBDA: Cannabidiolic acid; CBDV: Cannabidivarin; CBG: Cannabigerol; CBGA: Cannabigerolic acid; CBL: Cannabicyclol; CBN: Cannabinol; ChBL: Cherry Blossom; CS: Calibration standards; CT1: Cherry x T1; CW: Cherry Wine; GC: Gas chromatography; ICH: International Council for Harmonization; IS: Internal Standard; LC: Liquid chromatography; QC: Quality control; THCA: Δ -9-Tetrahydrocannabinolic acid; THCV: Tetrahydrocannabivarin; UF/IFAS: University of Florida's Institute of Food and Agricultural Science; UPLC-MS/MS: Ultra-performance liquid chromatography-tandem mass spectrometry; USDA: United States Department of Agriculture

Acknowledgements

The authors would like to acknowledge Dr. Bonnie A. Avery for initiating involvement in the Industrial Hemp Pilot Project. The authors gratefully acknowledge Jerry Fankhauser for providing logistics support and Aaron Reyes for his support in sample preparation.

Authors' contributions

ECB drafted the manuscript, acquired sample data, and performed data analysis. RY and JHF cultivated, dried, and ground samples for analysis. AS,

SHK, SRK, TIK, and RP supported method development. All authors read and approved the final manuscript.

Funding

This study was supported by the University of Florida Clinical and Translational Science Institute, which is supported in part by the NIH National Center for Advancing Translational Sciences under award number UL1TR001427.

The cultivation study and hemp material collection were conducted as part of the UF/IFAS Industrial Hemp Pilot Project funded by Green Roads West, LLC. Green Roads West, LLC is a CBD company. No authors have affiliation with Green Roads West, LLC and the company was not involved in the design, conduct, or decision to publish.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

N/A

Consent for publication

N/A

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL, USA. ²North Florida Research and Education Center, University of Florida, Quincy, FL, USA. ³Translational Drug Development Core, Clinical and Translational Science Institute, University of Florida, Gainesville, FL, USA. ⁴Tropical Research and Education Center, University of Florida, Homestead, FL, USA. ⁵Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA.

Received: 6 May 2020 Accepted: 28 October 2020

Published online: 10 December 2020

References

- The Agricultural Improvement Act of 2018, S.3042, 115th Congress of the United States. 2018. <https://www.congress.gov/bills/115th-congress/house-bill/2/text>. Accessed 20 April 2020.
- Aizpurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxebarria N, Usobiaga A. Identification and quantification of cannabinoids in Cannabis sativa L. plants by high performance liquid chromatography-mass spectrometry. *Anal Bioanal Chem*. 2014;406(29):7549–60.
- Andre CM, Hausman JF, Guerriero G. Cannabis sativa: the plant of the thousand and one molecules. *Front Plant Sci*. 2016;7:19.
- Citti C, Braghieri D, Vandelli MA, Cannazza G. Pharmaceutical and biomedical analysis of cannabinoids: a critical review. *J Pharm Biomed Anal*. 2018;147:565–79.
- Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, Woodcock H, Dorward P, Pigliacampo B, Close S, Platt B, Riedel G. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), Delta(9)-tetrahydrocannabinol (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)*. 2012;219(3):859–73.
- The Establishment of a Domestic Hemp Production Program, 2019-23749, USDA, 84 FR 58522. 2019. <https://www.federalregister.gov/documents/2019/10/31/2019-23749/establishment-of-a-domestic-hemp-production-program>. Accessed 20 April 2020.
- Guidance Procedures 2.0. US Hemp Authority, Ed. 2019. <https://www.ushempauthority.org/guidance-procedures-2-0/>. Accessed 20 Apr 2020.
- Gul W, Gul SW, Chandra S, Lata H, Ibrahim EA, ElSohly MA. Detection and quantification of cannabinoids in extracts of cannabis sativa roots using LC-MS/MS. *Planta Med*. 2018;84(4):267–71.
- Gul W, Gul SW, Radwan MM, Wanas AS, Mehmedic Z, Khan II, Sharaf MH, ElSohly MA. Determination of 11 cannabinoids in biomass and extracts of different varieties of cannabis using high-performance liquid chromatography. *J Aoac Int*. 2015;98(6):1523–8.
- Hanus LO. Pharmacological and therapeutic secrets of plant and brain (endo)cannabinoids. *Med Res Rev*. 2009;29(2):213–71.
- Hemp/CBD in Florida. Florida Department of Agriculture and Consumer Services., Ed. 2020. <https://www.fdacs.gov/Cannabis-Hemp/Hemp-CBD-in-Florida>. Accessed 20 Apr 2020.
- Hemphill JK, Turner JC, Mahlberg PG. Cannabinoid Content of individual plant organs from different geographical strains of Cannabis Sativa L. *J Nat Prod*. 1980;43(1):112–22.
- Iffland K, Carus M, Grotenhermen F. Decarboxylation of Tetrahydrocannabinolic acid (THCA) to active THC. *European Industrial Hemp Association*; 2016.
- Industrial Hemp Technical Manual Version 3, Health Canada Office of Controlled Substances, 2004. Available from: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/hc-ps/alt_formats/hecs-sesc/pdf/pubs/precurs/hemp-induschanvre/tech-man/hemp-tech-manual-eng.pdf.
- Jikomes N, Zoorob M. The cannabinoid content of legal Cannabis in Washington State Varies systematically across testing facilities and popular consumer products. *Sci Rep*. 2018;8(1):4519.
- Leghissa A, Hildenbrand ZL, Schug KA. A review of methods for the chemical characterization of cannabis natural products. *J Sep Sci*. 2018;41(1):398–415.
- Nahar L, Guo M, Sarker SD. Gas chromatographic analysis of naturally occurring cannabinoids: a review of literature published during the past decade. *Phytochem Anal*. 2020b;31(2):135–46.
- Nahar L, Onder A, Sarker SD. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). *Phytochem Anal*. 2020a;31(4):413–57.
- Nie B, Henion J, Ryona I. The role of mass spectrometry in the cannabis industry. *J Am Soc Mass Spectrom*. 2019;30(5):719–30.
- Office of Regulatory Affairs Laboratory Manual Volume II. Estimation of measurement of uncertainty. Rockville: Office of Regulatory Affairs, Food and Drug Administration; 2019. Available from: <https://www.fda.gov/media/73928/download>.
- AOAC Official Method 2019.003. Standard Method Performance Requirements (SMPRs) for Quantification of Cannabinoids in Plant Materials of Hemp (Low THC Varieties Cannabis sp.), Association of Official Analytical Chemists. 2019. Available from: https://www.aoc.org/wp-content/uploads/2019/10/SMPR-2019_003.pdf.
- Vaclavik L, Benes F, Fenclova M, Hricko J, Krmela A, Svobodova V, Hajslava J, Mastovska K. Quantitation of Cannabinoids in cannabis dried plant materials, concentrates, and oils using liquid chromatography–diode array detection technique with optional mass spectrometric detection: single-laboratory validation study, first action 2018.11. *J Aoac Int*. 2019;102(6):1822–33.
- ICH Harmonised Tripartite Guideline. Q2(R1): Validation of Analytical Procedures: Text and Methodology. <http://www.ich.org/LOB/media/MEDIA417.pdf>. Accessed 20 April 2020.
- Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, van Antwerp J, Parcher JF, ElSohly MA, Khan IA. Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. *Cannabis Cannabinoid Res*. 2016;1(1):262–71.
- Yang R, Berthold EC, McCurdy CR, da Silva Benevenuto S, Brym ZT, Freeman JH. Development of cannabinoids in flowers of industrial hemp (Cannabis sativa L.): a pilot study. *J Agric Food Chem*. 2020;68(22):6058–64.
- Zivovinic S, Alder R, Allenspach MD, Steuer C. Determination of cannabinoids in Cannabis sativa L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. *J Anal Sci Technol*. 2018;9(1):27.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/338905196>

First Report of Cercospora Leaf Spot Caused by *Cercospora* cf. *flagellaris* on Industrial Hemp in Florida

Article in *Plant Disease* · January 2020

DOI: 10.1094/PDIS-11-19-2287-PDN

CITATIONS

0

READS

87

4 authors, including:



Marcus Vinicius Marin

University of Florida

27 PUBLICATIONS 46 CITATIONS

[SEE PROFILE](#)



Johan Desaegeer

University of Florida

64 PUBLICATIONS 481 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Soil fumigation [View project](#)



Non-fumigant chemical and biological nematicides for vegetable, small fruit, and alternative crop production in Florida [View project](#)

First Report of Cercospora Leaf Spot Caused by *Cercospora* cf. *flagellaris* on Industrial Hemp in Florida

M. V. Marin, J. Coburn, J. Desaeger and N. A. Peres[†], University of Florida, Wimauma FL 33598. [†]Corresponding author: nperes@ufl.edu

During a greenhouse (July to September) and a field trial (October to December) in 2019, leaf spots were observed on up to 60% of leaves of hemp plants (*Cannabis sativa*). Symptoms started on older leaves and eventually spread throughout the canopy. Infections began with small yellow, individual flecks. Lesions developed to turn light tan, or even white, with yellow halos and fascicles of conidiophores were visible to naked eye at the center. Severely infected leaves usually developed chlorosis (yellowing), which lead to premature defoliation. Diseased leaves were surface sterilized with 10% bleach solution for 90 seconds and isolation was performed on General Isolation (GI) medium (Forcelini et al. 2016). Resulting colonies were whitish to gray after incubation in a growth chamber at 25°C, 12/12 photoperiod. Isolates were single-spored and resulting colonies were transferred to carrot-agar (CA) and PDA+6% sucrose where they appeared brown- to- dark color due to sporulation (Figure 1) (Leslie and Summerell 2006). Three isolates were selected for identification and pathogenicity tests. Conidiophores were brown, straight or geniculate, uniform in width, multi-septate, born in fascicles of five to twelve on the abaxial portion of the leaf, and ranged from 300 to 700 μm (Avg=560 μm , n=25). Solitary conidia were hyaline, slightly curved or straight, needle-shaped, truncate at the base and terminal at the tip, with indistinct septa ranging from three to fifteen, and size ranging from 60 to 240 μm (Avg=180 μm , n=25).

24 DNA was extracted from the same three isolates using FastDNA kit (MP Biomedicals,
 25 Solon, OH) and the ribosomal internal transcribed spacer (ITS) regions 1 and 2, and
 26 actin region (ACT) were sequenced using primer pairs ITS4/ITS6 (White et al. 1990),
 27 and ACT-512F/ACT-783R (Weir et al. 2012), respectively. Sequences were deposited
 28 in the GenBank as accession numbers MN633273-MN633275, and MN635543-
 29 MN635545, for ITS, and ACT, respectively. BLAST query of the sequences matched
 30 *Cercospora cf. flagellaris*, with 99 to 100% identity (GenBank accession no. MK989497,
 31 and KX443853 for ITS, and ACT, respectively). To fulfill Koch's postulates, isolates
 32 were grown for 20 days on CA in a growth chamber (25°C, 12/12 photoperiod). Spores
 33 were harvested using sterile water plus 0.01% Tween 20, and the suspension was
 34 adjusted to 10⁴ spores/ml. Three-week-old potted plants of 'Yuma-2' and 'Carmagnola
 35 Selezionata' were inoculated by spraying the spore suspension until run-off. Plants were
 36 incubated in a moist chamber for 72h, and maintained in the greenhouse. Control plants
 37 were sprayed with sterile water and kept under the same conditions. Three weeks after
 38 inoculation, all inoculated plants had *Cercospora* leaf spots, whereas the controls
 39 remained healthy. Five plants of each cultivar were used per isolate, and two of each as
 40 controls, and pathogenicity tests were repeated once. The same pathogen was re-
 41 isolated from the inoculated plants. *Cercospora cf. flagellaris* has a wide host range with
 42 reports on plant species from 24 different families. In Kentucky, increasing acreage of
 43 hemp fields resulted in recent reports of this pathogen (Albu et al. 2016; Doyle et al.
 44 2019). To our knowledge, this is the first report of *C. cf. flagellaris* causing leaf spots on
 45 hemp in Florida. Hemp can be used for fiber, building materials, forages, human food
 46 products, and oil extraction for pain relief, and could be a valuable alternative crop for

47 Florida. To support the future viability and sustainability of the crop in Florida, more
48 work is needed to assess the epidemiology, cultivar response, fungicide sensitivity, and
49 management of this disease.

50

51 **References:**

52 Albu, S. et al. 2016. Phytopathology, 106:1376-1385.

53 Doyle, V. P., et al. 2019. Plant Disease, 103: 1784.

54 Forcelini, B. B. et al. 2016. Plant Disease, 100: 2050-2056.

55 Leslie, J.F. and Summerell, B.A. 2006. The Fusarium Laboratory Manual. Blackwell
56 Publishing Ames, Hoboken.

57 Weir, B. S., Johnston, P. R., & Damm, U. 2012. Studies in mycology, 73: 115-180.

58 White, T. J., et al. 1990. PCR protocols: A Guide to Methods and Application. Academic
59 Press, San Diego, CA.

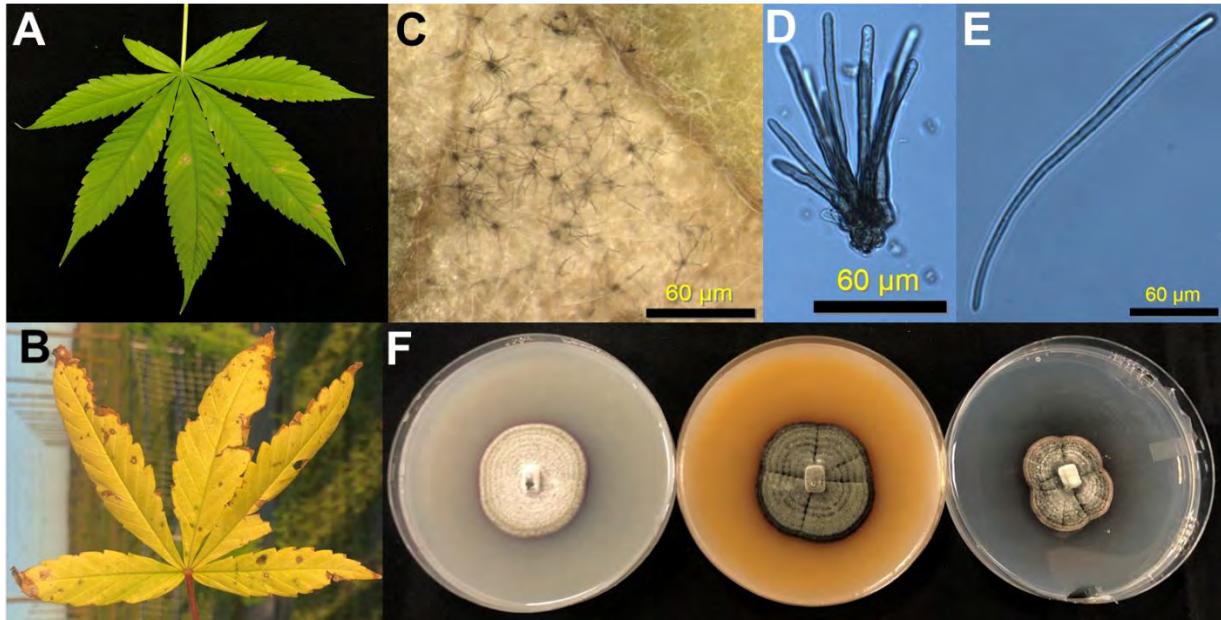


Figure 1. (A) Initial infection of *Cercospora cf. flagellaris* on hemp leaf with small yellow, individual flecks. (B) Severely infected leaves with chlorosis (yellowing), which leads to premature defoliation. (C) Conidiophores bearing conidia on symptomatic tissue. (D) Fascicle of conidiophores. (E) Filiform conidia. (F) Morphology of 15-day-old colonies on PDA (left), carrot agar (middle), and PDA+6% sucrose (right).

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/342479145>

First Report of *Curvularia pseudobrachyspora* Causing Leaf Spot on Hemp (*Cannabis sativa*) in Florida

Article in *Plant Disease* · June 2020

DOI: 10.1094/PDIS-03-20-0546-PDN

CITATIONS

0

READS

61

5 authors, including:



Marcus Vinicius Marin

University of Florida

27 PUBLICATIONS 46 CITATIONS

[SEE PROFILE](#)



Nan-Yi Wang

University of Florida

19 PUBLICATIONS 210 CITATIONS

[SEE PROFILE](#)



Johan Desaeger

University of Florida

64 PUBLICATIONS 481 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Non-fumigant chemical and biological nematicides for vegetable, small fruit, and alternative crop production in Florida [View project](#)



Emerging plant pathogens [View project](#)

**First Report of *Curvularia pseudobrachyspora* Causing Leaf Spot on Hemp
(*Cannabis sativa*) in Florida**

M. V. Marin, N.-Y. Wang, J. Coburn, J. Desaeger and N. A. Peres[†], University of
Florida, Wimauma FL 33598. [†]Corresponding author: nperes@ufl.edu

Hemp (*Cannabis sativa* L.) is an emerging crop in Florida, with potential use in a variety of commercial and industrial products, including rope, textiles, bioplastics, and insulation. During a field trial in 2019 (October to December) in Wimauma, FL, leaf spots were observed on up to 70% of one-month-old hemp plants on several varieties, such as 'Pumma-2', 'Eletta Campana', 'Carmagnola Selezionata', and 'Tygra' with up to 50% leaf damage in the field. Symptoms started on young and old leaves with small yellow spots that eventually turned tan to brown with a yellow halo. Pieces of diseased leaf tissue were surface sterilized with a 10% bleach solution for 90 s, rinsed twice with sterile deionized water, and then placed on General Isolation medium (Forcelini et al. 2016). The plates were kept in a growth chamber at 25°C under a 12/12 photoperiod. Fungal colonies with sparse aerial mycelium, fimbriate margins, and pale light gray zones or alternate gray olivaceous-to-brown zones on the surface were consistently isolated and single-spored. Four isolates were selected for identification and pathogenicity tests. Conidia measured (n = 25) ranged from 20.3 to 31.7 (average = 26.4; SD = 2.8) µm long and from 9.5 to 15.9 (average = 12.9; SD = 1.3) µm wide and were borne in groups from the apex of geniculate conidiophores and mostly curved, ellipsoidal to obovoid, pale brown to brown with 2 to 3 distoseptate and middle cells larger and darker than the others. These morphological characteristics were similar to

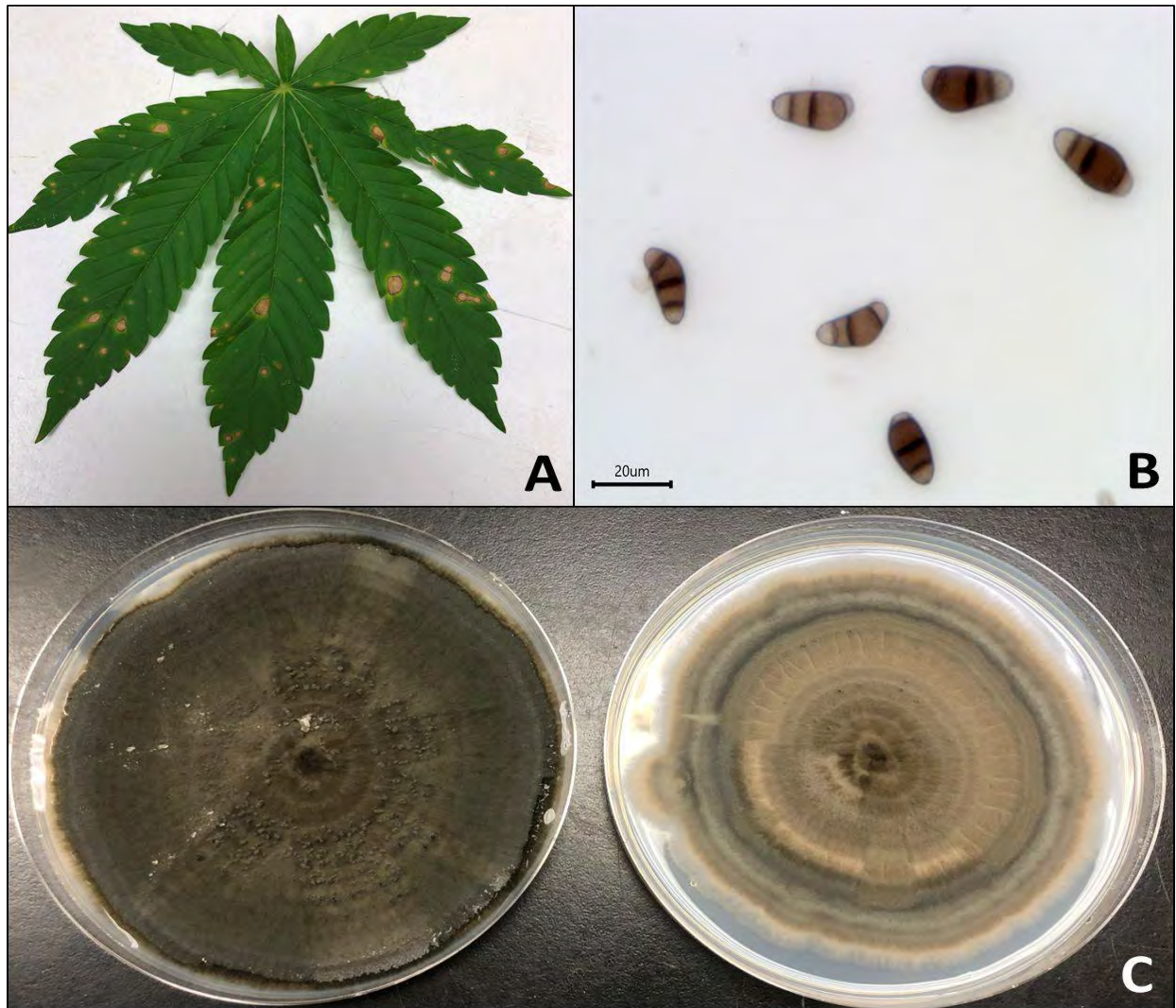
24 *Curvularia* spp. (Tan et al. 2018). DNA was extracted from the same four isolates using
 25 the FastDNA kit (MP Biomedicals, Solon, OH) and the ribosomal internal transcribed
 26 spacer (ITS) region, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and
 27 translation elongation factor 1-alpha (EF1- α) genes were amplified following the method
 28 by Tan et al. (2018), sanger sequenced by Genewiz (South Plainfield, NJ), and
 29 deposited in GenBank (accession no. MT071994 and MT072019-MT072021,
 30 MT085362-MT085365, MT085366-MT085369 for ITS, GAPDH, and EF1- α ,
 31 respectively). BLASTn searches revealed isolates 19-407, 19-409, and 19-410 were
 32 99.6% identical to *C. pseudobrachyspora* CPC 28808 (99.32% for ITS, 99.58% for
 33 GAPDH, and 99.88% for EF1- α), whereas isolate 19-405 had 98.53 to 99.4% similarity
 34 to the same species. Phylogenetic analyses using maximum likelihood and Bayesian
 35 inference differentiated isolate 19-405 from the other three isolates, suggesting the
 36 possibility of a cryptic species. To fulfill Koch's postulates, spores of the same four
 37 isolates were harvested in 0.01% Tween 20, and the suspensions were adjusted to 10^5
 38 spores/mL. Four 5-week-old potted plants of 'Puma-3' per isolate were inoculated by
 39 spraying the spore suspension to run-off. Plants were kept inside a plastic bag for 72 h
 40 and maintained in the greenhouse. Control plants were sprayed with sterile deionized
 41 water and kept under the same conditions. The pathogenicity test was repeated once.
 42 Two weeks after inoculation, controls remained healthy, whereas all inoculated plants
 43 had leaf spots as described above and the pathogen was re-isolated from symptomatic
 44 tissue. To our knowledge, this is the first report of *C. pseudobrachyspora* causing leaf
 45 spot on hemp. *C. pseudobrachyspora* has been reported causing leaf spot on areca
 46 palm (*Areca catechu*) in China (Wang et al. 2019) and on goosegrass (*Eleusine indica*)

in Thailand (Marin-Felix et al. 2017). Goosegrass is a common annual weed found throughout Florida (Buker et al. 2002.) and might serve as an alternative host in the absence of hemp. More studies are needed to understand the epidemiology of this disease and foster disease management programs in Florida.

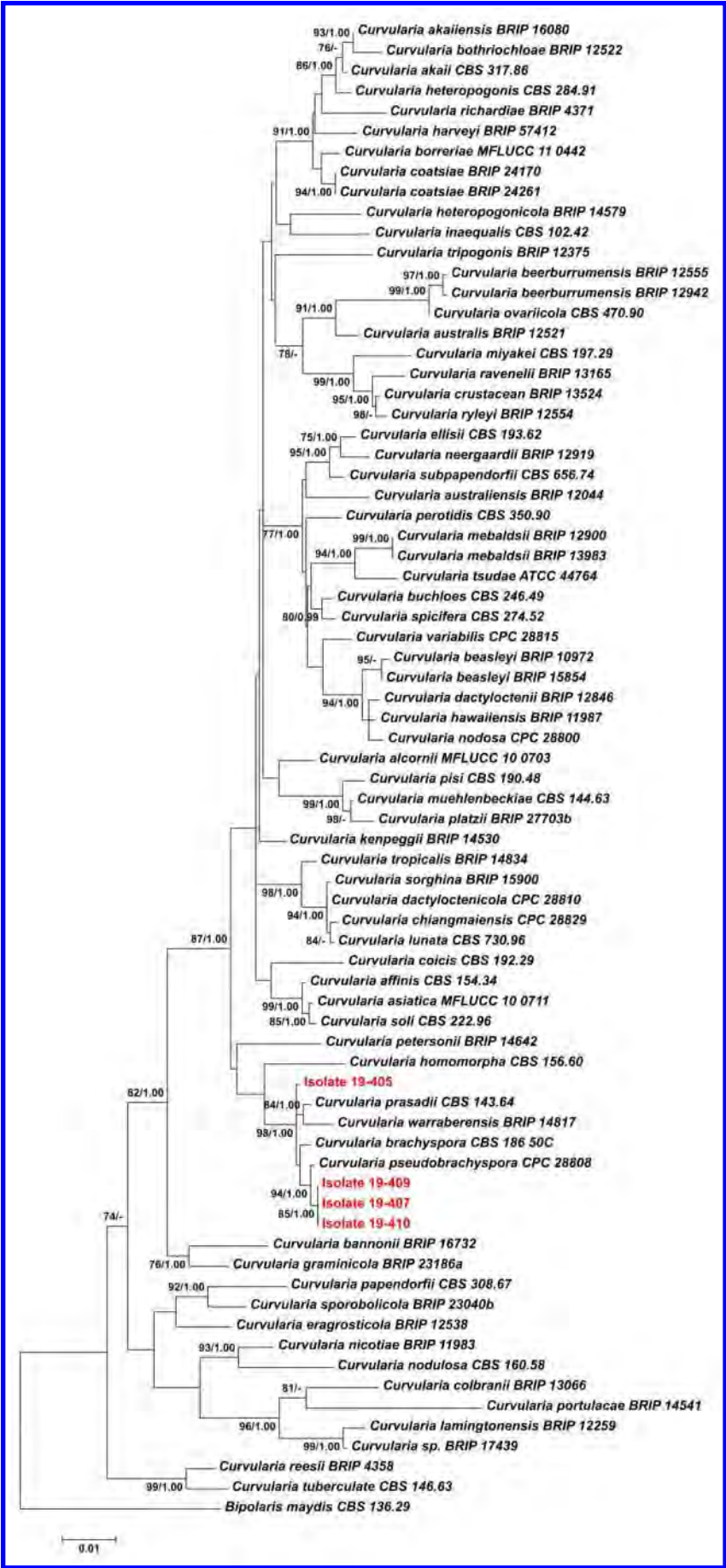
References:

- Buker, R. S., et al. 2002. Weed Technol. 16:309. [https://doi.org/10.1614/0890-037X\(2002\)016\[0309:CACOAP\]2.0.CO;2](https://doi.org/10.1614/0890-037X(2002)016[0309:CACOAP]2.0.CO;2)
- Forcelini, B. B., et al. 2016. Plant Dis. 100:2050. <https://doi.org/10.1094/PDIS-01-16-0118-RE>
- Marin-Felix, Y. et al., 2017. Mycosphere 8:1556. <https://doi.org/10.5943/mycosphere/8/9/11>
- Tan, Y. P., et al. 2018. MycoKeys 35:1. <https://doi.org/10.3897/mycokeys.35.25665>
- Wang, H., et al. 2019. Plant Dis. 103:150. <https://doi.org/10.1094/PDIS-06-18-1096-PDN>

The author(s) declare no conflict of interest.



Supplementary Fig. S1. Symptoms on naturally infected leaves of hemp (*Cannabis sativa*) by *Curvularia pseudobrachyspora* (A). Conidia (B) and morphology of 10-day-old colonies on potato dextrose agar (C; left, isolate 19-405; right, isolate 19-407).



Supplementary Fig. S2. Phylogenetic tree estimated from the concatenated sequences of the ribosomal internal transcribed spacer (ITS) region, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and translation elongation factor 1-alpha (EF1- α) genes using maximum likelihood and Bayesian inference methods. Isolates 19-405, 19-407, 19-409, and 19-410 are those described in this study. Bootstrap values greater than 70% (1,000 replications) and posterior probability greater than 0.95 are given at the nodes. The scale bar indicates 0.01 substitution per nucleotide position.

First Report of *Diaporthe phaseolorum* Causing Stem Canker of Hemp (*Cannabis sativa*)

M. V. Marin, N.-Y. Wang, J. D. Coburn, J. Desaeger and N. A. Peres[†], University of Florida, Wimauma FL 33598. [†]Corresponding author: nperes@ufl.edu

Hemp is an annual herbaceous plant that is used for its fiber and oil in a variety of commercial and industrial products. In Florida, it is currently being explored as a new specialty crop. During a field trial from October to January 2019 in Wimauma, FL, a stem canker was observed on up to 60% of three-month-old plants of 'Eletta Campana', 'Carmagnola Selezionata', and 'Tygra'. Symptoms started on the main stems with light-to-dark brown lesions of different sizes and shapes. Over time, the lesions coalesced into large necrotic areas and bore pycnidia. Isolations were made from diseased stem tissues on General Isolation medium (Amiri et al. 2018) after surface disinfestation (Marin et al. 2020). The plates were placed in a growth chamber at 25°C under a 12/12 photoperiod. A fungus with white, floccose, aerial mycelium and pycnidia producing alpha and beta conidia was consistently isolated. Three single spore isolates were chosen for identification and pathogenicity tests. Pycnidia on PDA were globose to irregular and ranged from 170 to 250 µm long (210 ± 2.5 , $n = 50$) and 140 to 220 µm wide (180 ± 2.7 , $n = 50$). The alpha conidia were unicellular, hyaline, ellipsoidal to fusiform and ranged from 5.3 to 7.7 µm long (6.5 ± 1.6 , $n = 50$) and 1.5 to 4.6 µm wide (2.8 ± 1.8 , $n = 50$). The beta conidia were hyaline, elongated, filiform, straight or curved and ranged from 10.2 to 17.7 µm long (16.1 ± 2.2 , $n = 50$) and 0.5 to 1.8 µm wide (0.8 ± 0.2 , $n = 50$). Perithecia were not observed. Based on morphological features, the fungus

24 was similar to anamorphs of *Diaporthe* spp. (Santos et al. 2011; Udayanga et al. 2015).
 25 DNA from the same three isolates was extracted using the FastDNA kit, and the
 26 ribosomal internal transcribed spacer (ITS), β -tubulin (TUB), and calmodulin (CAL)
 27 regions were amplified following Udayanga et al. (2014), and Sanger sequenced by
 28 Genewiz. Sequences were deposited in GenBank (accession no. MT497039 to
 29 MT497047 for ITS, TUB, and CAL). BLASTn searches revealed isolates 20-58, 20-59,
 30 and 20-60 were 96.34% identical to the epitype isolate *D. phaseolorum* AR4203 for ITS
 31 (KJ590738.1, 527 bp out of 547 bp), 100% for TUB (KJ610893.1, 459 bp out of 459 bp),
 32 and 100% for CAL (KJ612135.1, 522 bp out of 522 bp) (Udayanga et al. 2015). Their
 33 identity was confirmed by phylogenetic analyses using maximum likelihood and
 34 Bayesian inference methods. To complete Koch's postulates, pycnidia of the same
 35 three isolates were harvested and crushed in 2 ml Eppendorf tubes containing 0.01%
 36 Tween 20. Conidia suspensions were adjusted to 10^6 spores/ml. Three 5-week-old
 37 potted plants of 'Eletta Campana' and 'Carmagnola Selezionata' per isolate were
 38 inoculated using a 1 ml syringe with a needle by injecting 200 μ l of the suspension into
 39 the stem. Plants were placed inside clear plastic bags for 48 h and maintained in the
 40 greenhouse. Control plants were injected with sterile deionized water and kept under
 41 the same conditions. The pathogenicity test was repeated once. Four weeks after
 42 inoculation, inoculated plants developed stem cankers from which the same pathogen
 43 was isolated, whereas controls remained healthy. To our knowledge, this is the first
 44 report of *D. phaseolorum* causing stem canker on hemp. This pathogen has been
 45 reported causing canker on sunflower and *Phaseolus* spp. (Gomzhina and Gannibal
 46 2018; Udayanga et al. 2015; Vrandecic et al. 2009). This discovery may help shape

47 future research into disease epidemiology and management for a crop in which very
 48 limited disease information is available at the moment.

49

50 **References:**

51 Amiri, A., et al. 2018. Plant Dis. 102:2142. <https://doi.org/10.1094/PDIS-11-17-1810-RE>

52 Gomzhina, M. M., and Gannibal, P. B. 2018. Microbiol. Indep. Res. J. 5:65.

53 <https://doi.org/10.18527/2500-2236-2018-5-1-65-70>

54 Marin, M. V., et al. 2020. Plant Dis. 104:1536. <https://doi.org/10.1094/PDIS-11-19-2287->

55 [PDN](#)

56 Santos, J. M., et al. 2011. Persoonia 27:9. <http://dx.doi.org/10.3767/003158511X603719>

57 Udayanga, D., et al. 2014. Persoonia 32:83. <https://doi.org/10.3767/003158514X679984>

58 Udayanga, D., et al. 2015. Fungal Biol. 119:383.

59 <http://dx.doi.org/10.1016/j.funbio.2014.10.009>

60 Vrandecic, K., et al. 2009. Plant Dis. 93:1074. <https://doi.org/10.1094/PDIS-93-10->

61 [1074C](#)

62

63 The author(s) declare no conflict of interest.

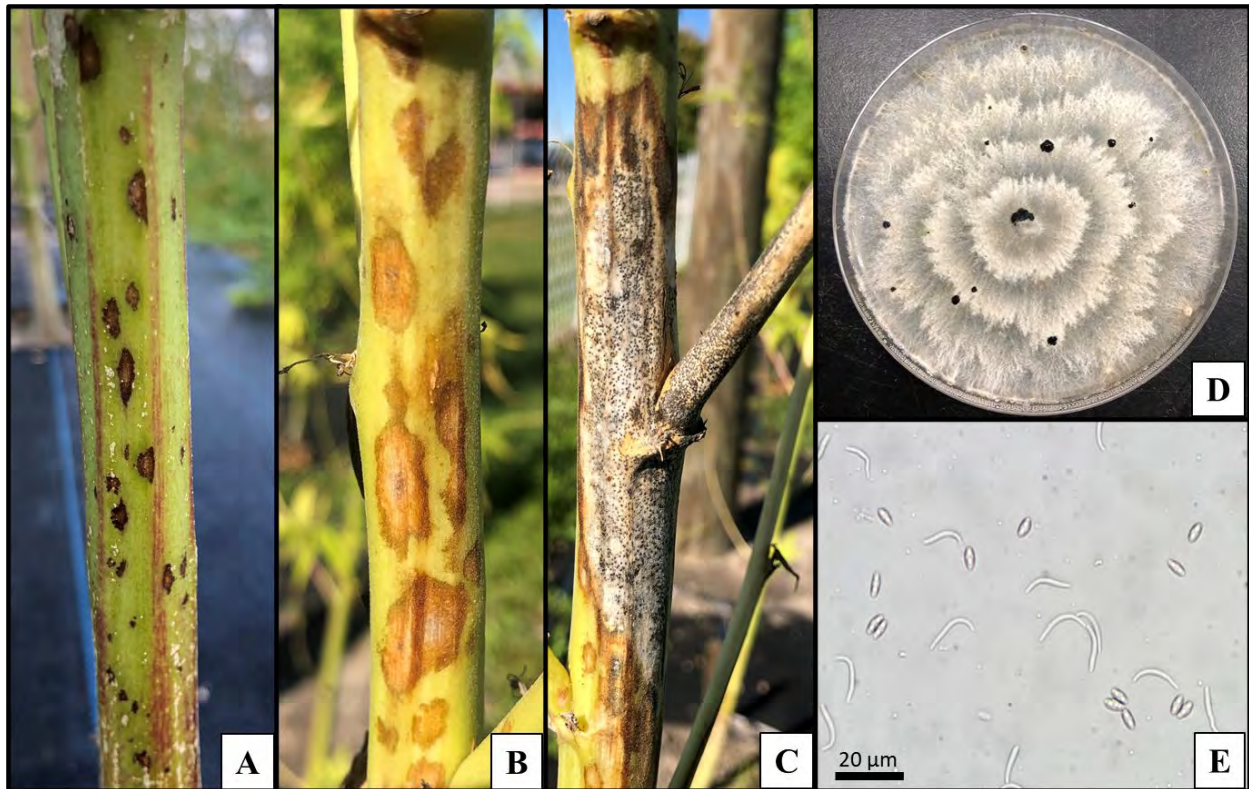


Figure 1. (A-C) Symptoms in different stages of disease development of hemp stem canker, caused by *Diaporthe phaseolorum* on 'Eletta Campana'; (D) Two-weeks-old colony grown on PDA; and (E) alpha- and beta-conidia.

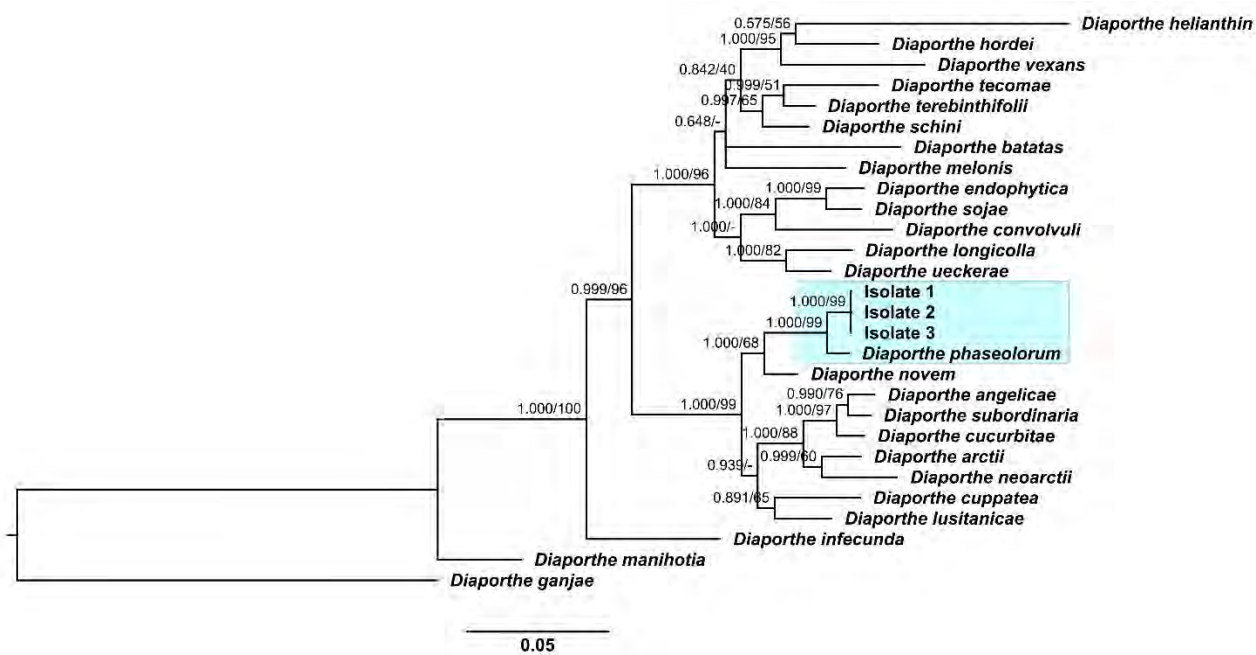


Figure 2. Phylogenetic tree estimated from the concatenated sequences of the ribosomal internal transcribed spacer (ITS) region, beta-tubulin (TUB), and calmodulin (CAL) genes using partitioned maximum likelihood and Bayesian inference methods with GARLI 2.0.1 and MrBayes 3.2.7, respectively. The reference taxa employed in the phylogenetic analysis were retrieved from GenBank according to Udayanga et al. (2015). Isolates 1 (20-58), Isolate 2 (20-59), and Isolate 3 (20-60) are those described in this study. Posterior probabilities and bootstrap support values (1,000 replicates) are given at the nodes. The scale bar indicates 0.05 substitutions per nucleotide position.

DDRE-14. HEMP DERIVED EXTRACELLULAR VESICLES (EVS): A POTENTIAL ANTI-GLIOMA THERAPY

Hassan Azari, Nasser Nassiri Koopaei, Mohammad-Zaman Nouri, Jesse D Hall, Nancy D Denslow, Thomas D Schmittgen, Brent A Reynolds, University of Florida

Neuro-Oncology, Volume 22, Issue Supplement_2, November 2020, Page ii64,
<https://doi.org/10.1093/neuonc/noaa215.259>

Published: 09 November 2020

Hassan Azari, Nasser Nassiri Koopaei, Mohammad-Zaman Nouri, Jesse D Hall, Nancy D Denslow, Thomas D Schmittgen, Brent A Reynolds, DDRE-14. HEMP DERIVED EXTRACELLULAR VESICLES (EVS): A POTENTIAL ANTI-GLIOMA THERAPY, *Neuro-Oncology*, Volume 22, Issue Supplement_2, November 2020, Page ii64, <https://doi.org/10.1093/neuonc/noaa215.259>

INTRODUCTION

Extracellular vesicles (EVs) have been harvested from many plant sources, some of which have anti-cancer effects and some could be used as therapeutic nanodelivery vectors. Hemp plant is a natural source of cannabinoids, of which delta 9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have proven anti-cancer properties.

HYPOTHESIS

We hypothesized that hemp EVs are enriched in cannabinoids and their application will reduce glioblastoma (GBM) tumor progression.

APPROACH

EVs were isolated from the hemp plant using ultracentrifugation. Nanotracking analysis, electron microscopy and liquid chromatography tandem mass spectrometry (LC-MS/MS) were utilized to characterize EVs. GBM cell lines were cultured in the neurosphere assay to evaluate hemp EVs anti-glioma effects. Fluorescent-labelled EVs were used to evaluate their brain tissue distribution in orthotopic patient-derived GBM xenografts.

RESULTS

Hemp EVs have a median diameter of 112.6nm with a typical lipid-bilayer structure. LC-MS/MS have shown that while cannabidiolic, cannabigerolic, and tetrahydrocannabinolic acids represent $69.1 \pm 2.1\%$, $19.1 \pm 1.6\%$, $6.5 \pm 0.54\%$ of the total cannabinoids in hemp EVs, CBD and THC only make $4.75 \pm 0.26\%$, and $0.5 \pm 0.3\%$. Hemp EVs are potent anti-glioma agents with a 7-day LD-50 of $1.04\mu\text{M}$ and $2.4\mu\text{M}$ [based on EVs total cannabinoid content] for KR-158 and L0 GBM lines, respectively. Compared

to the vehicle, overnight incubation of L0 cells with 1 μ M hemp EVs significantly reduced GBM cell migration (630.3 ± 61.43 vs 143.7 ± 8.7). Intranasal administration of hemp EVs led to a widespread distribution in tumor bearing brain including GBM tumor core.

CONCLUSION

Based on these results, hemp EVs with enriched cannabinoid content exert antiglioma effect *in-vitro* and when delivered intranasally, are widely distributed throughout the brain and within the tumor of PDX animals. Further experiments are ongoing to address the impact of nasally-delivered hemp EVs on tumor progression and compare to the application of purified acidic cannabinoids.