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Short title: Hemp volunteer persistence

Emergence and persistence of volunteer hemp in southern Florida

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Abstract

Introductions of new crops can provide alternate market opportunities, but also pose ecological risks. New crops lack established management, have uncertain performance issues, and may become weedy in their introduced region. The introduction of hemp (*Cannabis sativa* L.) into southern Florida poses a unique introduction scenario because of the subtropical climate and no commercial production on record, unlike in other eastern and midwest United States. We assessed escape from cultivation for hemp by tracking establishment and reproduction of volunteer plants from the earliest modern hemp planting in Florida. Hemp is a weed across much of the United States matching its historical distribution and has been assessed to be of high invasion risk for Florida because of its biological attributes, history of escape, and colonization in other states and countries. We conducted monitoring of volunteer plants and a seed establishment experiment in southern Florida and found that hemp volunteer plants occurred in pulses over time, with variable and declining germination. Volunteer plants persisted for up to two years and appeared in areas that were disked and mowed according to USDA approved hemp crop termination procedures. In the seed establishment experiment, we found that hemp established in disturbed soils (~9% of seeds planted) and that mean plant heights and seed counts were positively related to soil disturbance and nutrient addition. These findings show that hemp plantings should be monitored for volunteer establishment, and containment plans should be in place to control the establishment of volunteer hemp plants in agricultural fields. Our study further illustrates the need for multi-year monitoring and repeat termination procedures to ensure containment of hemp volunteers. There was limited evidence of volunteer establishment in surrounding areas and on undisturbed land. However, seed containment, equipment cleaning, and the monitoring of nearby fields and seed transportation routes remains warranted.

Keywords: *Cannabis sativa*, emergence, germination, seed regeneration, crop ecology

Management Implications

It is essential for hemp producers to adequately control the distribution and storage of seed (an oil-rich achene), especially for seed-crop type, but also for fiber-type hemp varieties. Often seeds labeled fiber-type will grow as seed-type in southern latitudes. Escape from cultivation and invasion risk in natural areas should be considered by all Florida hemp producers, especially those intending to operate at commercial scales with high potential for seed dispersal outside cultivation during transport and harvest. Hemp is predicted to have a high invasion risk in natural areas according to the UF/IFAS Assessment of Non-Native Plants in Florida's Natural Areas. This study demonstrates problematic volunteer hemp establishment and persistence. Florida Department of Agriculture and Consumer Services (FDACS 2020) rule 5B-57.014 declares hemp is "a potentially invasive plant species and is a threat to the plant life of this state if not properly controlled". The rule requires an environmental containment plan that defines site boundaries, equipment cleaning, and transportation precautions for each cultivation location. Based on our observations and experiments, hemp can persist outside of cultivation, though some form of soil disturbance and background nutrition appears to be required for establishment. Monitoring of fields and nearby areas should occur for at least two to three years after planting. Accidental seed dispersal by equipment and wildlife should be managed using on-site cleaning of equipment, secure storage in transit, and bird deterrence around harvest.

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Introduction

The introduction of hemp, varieties of *Cannabis sativa* L. that contain low levels of THC (less than 0.3% delta-9-tetrahydrocannabinol), into agricultural systems is increasing globally. The goal is to develop food, fuel, and fiber production and provide farms with additional crop and market opportunities (CPC 2023). Yet, hemp cultivation remains relatively limited across the United States (US). There is a risk that new hemp cultivation could lead to the plant becoming invasive, displacing native vegetation, and threatening native ecosystems (Canavan et al. 2022). Sometimes, agricultural weeds originate as crops, which then become feral when they regenerate voluntarily after harvest and persist (FISC 2023; Friedman et al. 2022). Eventually, they are considered weeds when they reduce the production and quality of other crops by competing for space, light, moisture, and nutrients (Warwick & Stewart 2005). To minimize the potential for new crops to escape cultivation, spread, and become environmental and agricultural weeds, invasion risk assessment is a critical step before introduction in a new region (Barney 2014; Dobbs et al 2022; Smith et al 2015). Hemp introduced to southern Florida poses a unique opportunity to investigate potential escape from cultivation because of the subtropical climate and lack of historical commercial production.

Hemp is an herbaceous annual native to parts of central and southern Asia (Clarke and Merlin 2013). In natural and cultivated settings with adequate soil structure, moisture and nutrition, it can reach heights of 0.3-6 m (Fike 2016; Kaur et al. 2023). Hemp has strong fibers that are durable and rot-resistant, making them ideal for textiles and material products (Small & Marcus 2002). Additionally, the plant is used to make foods from the seeds and cannabinoid-rich oils from flowers (Liu et al. 2017). Hemp seeds are achenes formed on inflorescences that provide abundant protein, healthy fats, and other nutrients and are often used in various foods and supplements (Vonapartis et al. 2015). Hemp flowers produce a resin with abundant secondary compounds known as cannabinoids, especially cannabidiol (CBD). Due to its many uses, it is one of the most widely introduced plants in the world and has adapted to a broad range of environmental conditions (Canavan et al. 2022). However, commercial cultivation in contemporary agriculture has been broadly limited because of its illegal status in many countries, owing to the psychoactive compounds of related varieties of cannabis used as recreational drugs. Since changes in U.S. federal laws in early 2014, there has been renewed interest in hemp as an

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alternative crop leading to increased commercial cultivation. Nevertheless, because of its often-complex legal status and potential for invasiveness, it has been heavily regulated in many states.

In the US, industrial hemp was reintroduced in 2014 and declared a commercial crop in 2018 following decades of control under drug enforcement laws (Addlesperger 2015; Mark et al. 2020; Rehman et al. 2021). As of 2021, 20,234 ha (50,000 ac) of hemp were cultivated in the US under permits split across fiber, seed, and flower production (Mark et al. 2020; USDA 2022). In 2021, the U.S. hemp industry was valued at $824,000,000, which included production in open fields, shade houses, and greenhouses (USDA 2022). Commercial planting in Florida began in 2020 with at most 100 ha approved for harvest by the FDACS (pers. comm., FDACS Division of Plant Industry).

In addition to economic and cultural considerations for hemp production, there have been concerns about escape from cultivation (Canavan et al. 2022). Many feral populations have originated from previous hemp cultivation in the US (Fig. 1), and seven midwest states have declared it a noxious weed (Filer 2020; USDOJ 1992; West 1998). During 1998-2006, the US Drug Enforcement Agency Domestic Cannabis Eradication and Suppression Program claimed to have destroyed more than 1.9 billion feral or wild "ditch weed" plants in the midwest (DEA 2018). Despite major eradication attempts, hemp populations have persisted in disturbed areas, such as along roads and waterways (Filer 2020).

It remains somewhat unclear the extent to which hemp could escape cultivation in Florida. Although Florida has no historical record of commercial hemp cultivation, eradication records and a predictive risk assessment have suggested that hemp poses an invasion risk to Florida's natural areas (Canavan & Flory 2019; Williams & Lieurance 2018). Feral cannabis plants have been documented in Florida, often as a result of seizures by law enforcement (FLMNH 2020; MBG 2023; Wunderlin et al. 2022). Decades of legal restrictions on cannabis possession seemingly minimized the distribution in Florida where records of feral populations otherwise remain sparse. Nevertheless, with intent to ensure proper control, commercial growers are required by state regulations to submit an environmental containment plan with the permit application (Florida Department of Agriculture and Consumer Services Rule 5B-57.014; FDACS 2020).

Research is needed to better understand the risk of hemp escape from cultivation and conditions that elevate invasion risk, especially in regions of recent commercial cultivation. We

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hypothesized that feral hemp populations can survive in low-nutrient environments, but they grow best in highly disturbed, nutrient-rich areas, such as abandoned agricultural fields (Small et al. 2003). We established outdoor plantings of hemp in southern Florida in 2019, followed by the studies presented herein to evaluate invasion risk including a systematic observation and a field experiment. Specifically, the studies include 1) a systematic observation related to volunteer population monitoring that quantified the plant emergence and seed production of volunteer hemp regenerating after cultivation, and 2) a field experiment that planted hemp seeds in an adjacent field under various nutrient and disturbance conditions. The experiments were designed to simulate a field that was abandoned after planting and a field edge where seed were spilled or discarded, respectively. Our overall goal was to better understand the potential for hemp to escape cultivation in agricultural areas by monitoring hemp germination, establishment, and persistence for succeeding generations.

Materials and Methods

Two independent research studies are described herein as ‘Volunteer population monitoring’ and ‘Seed establishment’. We began this work in 2020 following a 2019 research planting (i.e. ‘variety trial’) that resulted in volunteer seed establishment (Brym et al. 2020). We then developed an experiment to investigate the conditions under which hemp seed may establish outside of cultivation.

Site description

This research was conducted at the UF/IFAS Tropical Research and Education Center (TREC), Homestead, Florida (25.4687° N, 80.5007° W). The site was located within USDA hardiness zone 10b with a marine subtropical climate, an average annual temperature of 24°C (min 19°C, max 29°C), and 1473 mm/year of annual precipitation (FAWN 2020). Soil was described as Krome gravelly loam, which originates from limestone bedrock, and is well-drained, shallow (10-25 cm), calcareous (pH 7.4 - 8.0) and moderate in organic matter (3 - 10%; NCSS 1947; Nobel et al. 1996). Our research occurred in agricultural fields with historical soil disturbance and fertilizer applications.

Volunteer population monitoring

The site was first prepared and planted across several dates for a hemp variety trial in spring and summer 2019 without prior consideration for the post-harvest monitoring reported herein (Fig. 2). Monitoring volunteer populations began in 2020 after the termination of the variety trial in response to initial observations of volunteer establishment. To describe the formation of the variety trials, in early 2019, the site was mowed, disked, and plowed before planting. All plots received irrigation from overhead sprinklers for establishment, and fertility from broadcast slow-release granular fertilizer (Super Fruiter 6-13-16, Diamond R Co., Fort Pierce, FL) resulting in 125 kg N, 62 kg P_2O_5, and 336 kg K_2O per hectare. The variety trial involved 21 hemp varieties planted on up to four dates (May-July 2019) in a split plot design. Hemp seeds were sown 6 mm deep in raised furrows within 18.3 m x 30.5 m planting areas using an EarthWay seeder (#1001-B, EarthWay Products, Inc., Bristol, IN). Resulting plant densities were 60, 900, and 1500 seeds per plot for floral, grain, and fiber production types, respectively. Harvest was conducted 90-120 days after planting based on variety maturity in $1m^2$ sampling plots (20% experimental plot size) on varieties yielding an acceptable amount of seed or stem tissue for grain or fiber, respectively. The third and fourth planting date (~ 600 m^2) were terminated after minimal harvest by disk harrow per USDA hemp termination procedures (US Domestic Hemp Production Program 2021).

In January 2020, three months after completion of the variety trial, we began monitoring volunteer populations in the area of this earlier 2019 trial (~0.16 ha). All the plots, including those in the 2020 volunteer monitoring area, had propagule pressure resulting from seeds left in the field following the harvest. Here, plants outside the sample plots (80% of planted area) were left to drop seeds unassisted, akin to a field abandonment scenario whereby a producer plants hemp but does not harvest the crop due to such reasons as the crop tests out of compliance for harvest (>0.3% total THC) or there is a lack of access to a market for the crop and does not properly terminate. Monitoring for volunteers targeted plots of the first (~200 m^2) and second (~500 m^2) planting dates, which were among the best seed yielders of the 2019 trials (Fig. 2b). This combined area (~700 m^2) was evenly divided into two treatments, one of high and one of low propagule pressure (Fig. 2a). The low propagule pressure treatment had only the seeds in place deposited by the 2019 crops, while the high propagule pressure treatment also had a second addition of seeds. This enhanced seeding was intentionally created by translocating and

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dispersing seeds (~2 g/m^2, 'Han NE') from mature plants of the fourth (July) planting date to create a higher propagule pressure treatment. Few plants survived (n<10) from the July planting date in a plot of Han NE but they were uniquely productive for that planting date. Seeds were collected from all plants remaining in that plot, mixed, and distributed evenly across the high propagule pressure treatment. Because of differences in the sources and seeding events in each treatment, the proportion of seeds contributed by each variety, and possibly the varieties themselves, are believed to have differed between treatments. Potentially, all 21 varieties from 2019 may have seeded in the combined 700 m^2 observation area of 2020, though several primary candidates are likely due to proximity and reproduction that originated from China and southern Italy (Brym et al 2020). However, because we expect some seed mobility at shatter and did not track specific plots for volunteers, we cannot report directly on the varieties represented in the 2020 volunteer observations. Therefore, the abilities of specific varieties to become volunteer plants were also not observed.

The monitored area was not mowed or otherwise disturbed during observation. The rest of the field and surrounding area, acting as observational control, had either no hemp planted or hemp planted that was terminated by mowing and disking according to USDA-recommended best practices to prevent or reduce the emergence of hemp volunteer plants, and also sporadically mowed as routine to reduce weed dispersal. Hemp debris from other studies were routinely discarded as piles into the mowed and disked area as recommended for termination.

Volunteer hemp plants were left in the field, but counted and their sexes determined every 1 - 3 weeks in a 200 m^2 area. Bi-weekly data collection continued January - March 2020, with a pause until September 2020, then continued until the end of the year, followed by intermittent monitoring through 2021 and 2022. When the plants had mature seeds, heights were measured and whole plants were harvested. Seeds were extracted, counted, weighed, then redistributed back into their respective plots so as not to interrupt the volunteer population by sample removal.

Seed establishment experiment

In July 2020, we began a trial to better understand the conditions that permit discarded hemp seeds to establish and persist as volunteers. A fallow field (~800 m^2) was mowed but not tilled for six months before the trial. The experimental design was a randomized complete block

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design with treatment factors assigned to each of the 12 2-x-2-m^2 subplots. There were eight blocks (replications), each containing two rows of six plots (96 total subplots). Plots were separated by 2m wide buffers, which were mowed regularly. There were two nutrient treatment levels (high and low), two disturbance levels (high and low), and three varieties ('Helena, 'Han-NE', and 'CFX-1'; Table 1). The hemp varieties were selected to represent different primary uses of commercial hemp (dual, grain, fiber), cultivation origin (Italy, South China, Canada), sex (dioecious or monoecious), and variation in germination rate and seed size. Each of the resulting 12 possible combinations were represented once per block. For the nutrient treatment, 56 kg/ha of granular fertilizer (Super Fruiter 6-13-16, Diamond R Co., Fort Pierce, FL) was added to represent an agricultural field under standard cultivation, while the control treatment had no added nutrients. The disturbance treatment was rototilled 7 cm deep, while the control was left untilled and undisturbed.

All seeds planted in the seed establishment experiment originated from the same batch of seeds planted for the 2019 variety trial. One thousand live seeds (estimated by weight and germination rate) were sown into each plot. Seeds were broadcast in plots and lightly raked. Overhead irrigation was supplied for one week (until emergence).

Emergent seedlings were counted in each plot three days after planting, then daily for one week, or until no more seedlings emerged. Plant height was measured one month after planting, then at monthly intervals. When the plants had mature seeds, they were measured for height and harvested. In addition to redistributing some seeds back into plots, we retained seeds for germination tests.

Laboratory germination was conducted on harvested seeds. We tested a maximum of 60 seeds for each plot and harvest date. Petri dishes were prepared to hold up to 20 seeds placed on two sheets of paper towel in a modified "rag-doll" test (Dubeux 1999). Using a plastic pipette, 2 ml of deionized water was added to each dish, which was sealed using parafilm to prevent contamination and moisture loss. Dishes were placed in three stacks per harvest date in a growth chamber (I-35LL, Percival Co., Perry, IA) set at a constant 26°C with no light. Germination was recorded on day five assessing germination by a robust and healthy radicle. Radicles desiccating or diseased were not included in the count.

Statistical Analysis – Volunteer population monitoring

Mean numbers of seeds and plants for each collection date and treatment were evaluated. Statistical methods compared plant height and seed counts by analysis of variance and linear regression. Wilcoxon rank tests, via the function 'wilcox.text()' function in R were used to evaluate height and seed grouped by date and propagule pressure treatment.

Statistical Analysis – Seed establishment experiment

Mean numbers of seeds and plants for each collection date and treatment were evaluated. Statistical methods compared plant height and seed counts by analysis of variance and linear regression. Wilcoxon rank tests, via the function 'wilcox.text()' function in R were used to evaluate height and seed grouped by date, and block, nutrient, and variety. Because all resulting plants and seeds produced were from disturbed plots, disturbance level was not considered in the analyses. Confirming normality of data, an analysis of variance (ANOVA) was performed on seed and plant counts using the function 'aov()' with factors for block, nutrients, and varieties. Mean separation followed using a Tukey Post Hoc test via the function 'HSD.test()' in R.

Germination data was analyzed between treatments as the proportion of seeds germinating. An ANOVA was used with mean separation by two-sample t-tests, including a continuity correction (Luthria et al. 2008). All analyses and visualizations were conducted in R (R Core Team 2021).

Results and Discussion

Volunteer population monitoring

Volunteer hemp plants were observed within several months post-harvest, as 80% of the 2019 planted area was left to shatter and disperse as if abandoned (Fig. 3). More than 500 volunteer plants were observed within a 0.16 ha area, producing approximately 1500 seeds. The research trials included several varieties with vigorous biomass and seed productivity (Chinese and southern Italy), though it was not possible to determine specifically which varieties resulted in volunteer plants. Most varieties planted showed poor performance previously due to early flowering, low growth, and minimal seed set (Brym et al 2020), and for that reason, we expect the 500+ volunteer plants encountered to represent a relatively low number compared with what

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may result from a commercial setting with high plant density and vigorous seed production. The additional pulses of volunteers in following seasons may have originated from seed bank or volunteer reproduction. Seed quality decreased over time and volunteers did not persist in the abandoned field after two years. Seed bank and storage conditions with high temperatures and humidity are expected to lower seed germination over time (Clark et al. 1963), perhaps even more rapidly when directly exposed to open environments. Seed-eating herbivores such as rodents, birds, and beetles may have helped to reduce the numbers of viable hemp seeds in the soil. Hemp seeds appear larger than those of many locally common weeds (*Acalypha*, *Amaranthus*, *Euphorbia*, *Parthenium*, *Phyllanthus*, etc.), thus potentially rendering them more obvious and attractive to the herbivores. Coordinated timing of male and female flowering is also essential for hemp reproduction. Only a few individual volunteers produced the majority of seeds in seasonal pulses over a year suggesting the environment was challenging for growth and reproduction. Once the seeds germinated, density-dependent competition may have acted in patches with high seed shatter, but competition from plant residue and common weeds was likely more of a factor. Relatively few seeds with very poor germination quality points to the diminishing persistence of volunteer hemp.

The few volunteer plants observed outside the targeted monitoring area in mowed and tilled area was surprising because the USDA termination methods applied did not eliminate volunteers. All observed populations likely could have been terminated effectively with additional mowing and disking. The targeted monitoring areas without mowing and disking represented 50% of the total land acreage monitored yet produced nearly all the seed. Some plants were found in the mowed and disked area but did not produce seed in abundance. Of seed in the targeted monitoring area, the high propagule treatment produced 58% of the seeds while the low propagule treatment produced 42% (Figs. 4-5). We observed significantly more seeds from areas of high than low propagule pressure for the initial sampling period (Jan-Mar 2020, $p = 0.025$) and across all of 2020 ($p=0.014$; Figs. 4a, 5a-b). Volunteer plant counts also differed significantly between high (6.17 ± 0.38 SE) and low (4.46 ± 0.24 SE) propagule pressure areas ($p < 0.001$; Figs. 4c, 5c). Mean female plant height was 24.12 ± 3.47 SE cm for high propagule pressure and 13.96 ± 1.11 SE for low. The difference in female plant height was likely influenced by different varietal compositions in the high and low propagule pressure treatments.

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The high propagule pressure area was more likely to include individuals from vigorous varieties, or varieties that showed faster growth and higher seed production (i.e. Chinese origin). The highest mean number of mature seeds was observed on Mar 11, 2020, with 26.91 ± 0.82 SE seeds per ~5 m^2 in the high propagule pressure area; this was surprisingly in the spring season instead of as expected in the fall (Fig. 5a). Across dates, mean seed production per plant was 23.86 ± 2.99 SE for high propagule pressure and 14.06 ± 2.52 SE seeds for low (Fig. 5b). This is much lower than expected for plants in commercial production that can put on hundreds of seeds per individual (Kaur et al 2023). The highest mean number of volunteer plants was 10.67 ± 0.42 SE from the high propagule pressure plots on Jan 24, 2020 (~120 days after abandonment; Fig. 5c), also much less than commercial planting rates (50 plants/m^2). When plant counts were compared across propagule pressure with dates combined, we observed about twice as many plants in high as in low propagule pressure (Fig. 5b), accounting for the effect of additional high quality seed loads to the area. Plant height appeared as a useful indicator of reproductive potential where taller plants generally had greater seed production for the spring season January – March ($r^2 = 0.483$, Fig 4b), though the trend was not consistent across the year for January – December ($r^2 = 0.07$, $p = 0.007$, Fig. 5d).

The highest count, height, and seed production of volunteer hemp plants were encountered at the earliest stages of our study (January - March 2020). Following that period, no new plants were found until July and then again in September 2020. However, all the plants found in July 2020 were outside the targeted monitoring area in the area that was mowed and disked. From September to December 2020, there were very few plants that produced seeds (10.92 ± 6.79 SE seeds per 5m^2) and the majority came from high propagule pressure plots (80%). The two largest seed samples from Fall 2020 were taken in December (Figs. 5a,c). Although viable seeds presumably remained in the field seed bank, the plant population declined by the end of 2020. During March 2021, fewer than eight female flowering plants with mature seeds were observed in the field. Only a single male plant was found August 2021 with no additional observations by September 2021. Though we acknowledge a chance of seed bank, the field no longer contained volunteers and was returned to mowing and disking in a cover crop – fallow rotation. The combination of biomass build-up, weed competition, and lack of soil disturbance during the field abandonment simulation may have limited new plant establishment and reproduction. Yet, through associated activities with this study, even after long-term storage and likely poor-quality seeds, dried (55 ^{o}C for at least 72 hr) and stored seeds discarded in the monitoring area in January 2022 rapidly resulted in volunteer plants. This final observation of volunteer plants, in 2022, was likely incidental by the source unrelated to our monitoring study that we expected to be non-viable because of long-term storage in ambient conditions (Clark et al. 1963). Since then, the field was plowed and disked and no volunteer hemp plants have been observed.

Seed establishment

When hemp seeds were planted in an experiment on the edge of an agriculture field with disturbance and nutrient treatments, hemp grew in plots with nutrient addition and nutrient control but only in plots with soil disturbance (Table 2, Fig. 6). In the disturbed plots, plant height was significantly affected by nutrient, variety, and experimental block (Table 3). Plants that established and reproduced were still generally small with low seed count. Mean female plant height at harvest was 37.9 ± 1.97 SE cm for unfertilized and 48.3 ± 1.99 SE cm for fertilized plants (Table 2).

The total seed count across all five harvests was 706 seeds from 37 plants (Fig. 6a). Seed counts were observed only to be affected by total numbers of plants harvested ($p = 0.003$). Mean seed count was 42.78 ± 5.47 SE seeds/plant for unfertilized and 56.4 ± 7.12 SE seeds/plant for fertilized plants (Table 2, Fig. 6b). The proportion of seeds produced per nutrient treatment was not significantly different at 53% and 47% for high and low nutrients, respectively ($p = 0.82$). The proportion of plants produced per nutrient treatment was 32% and 42% for high and low nutrients, respectively, but not significantly different ($p = 0.69$, Fig. 6c). The proportion of total seeds produced by variety was significantly different at 58%, 32%, and 11% for 'Han-NE', 'Helena', and 'CFX-1', respectively (chi-square test; $p = 0.04$). The relatively good performance of 'Han-NE' in seed production was expected by observations of its subtropical latitude origin and its better performance in southern Florida across variety trials and complementary experiments (Table 1). Hence, differences in varietal factors such as cultivation type, origin, sex, and possibly seed size appeared to have affected proportions of seeds germinating in the seed establishment trial and germination test. The specific contribution of each of these factors and their interactions were beyond the scope of this study but are recommended for further investigation.

Seed germination tests reflected poor quality seed. Overall, seeds averaged 7.2% germination, with no significant difference between varieties and nutrient levels ($p = 0.72$). As noted, this poor germination quality combined with relatively few seeds subjected to consumption by herbivores and seedling competition with weeds and each other may have further lowered their numbers, leading to a reduced persistence of volunteer hemp. Continued monitoring should be conducted on and around hemp fields for the following years to observe such population decline or otherwise apply approved termination methods responsively for assurance.

Summary of research findings

Cannabis sativa has been observed and recorded growing in Florida (GBIF 2024), though origins of those plants and their persistence is unknown. Many cannabis plants were eradicated by law enforcement, and there is no evidence of sustained populations in Florida. Because hemp has a predicted high invasion risk from the literature (Williams and Lieurance 2018), it is essential to monitor and evaluate invasion risk before widespread introduction. FDACS rule 5B-57.014 declares hemp “a potentially invasive plant species and is a threat to the plant life of this state if not properly controlled” (FDACS 2021). The rule requires an environmental containment plan that defines site boundaries, equipment cleaning, and transportation precautions for each cultivation site (e.g., Barney 2014). Furthermore, the USDA has released best practices to prevent or reduce volunteer hemp that should be used following crop termination (US Domestic Hemp Production Program 2021). Yet, accidental distribution of seed and volunteer plants remains a significant concern for hemp at commercial scales.

Our research addressed on-farm volunteer monitoring and seed establishment at a closely monitored research site in southern Florida. Mature volunteer hemp plants were observed following plantings with populations that persisted across a calendar year. We observed that seeding rate positively affected volunteer counts and that seed establishment was positively affected by disturbance and fertilizer. Surprisingly, areas managed with USDA-recommended best practices were not sufficient in entirely preventing hemp volunteers. Ineffective containment and further escape from cultivation remains an ecological concern for agricultural and surrounding natural areas (Canavan et al. 2022; McKay et al. 2005).

Our observations suggest monitoring and containment practices are a critical aspect of hemp crop management with emphasis on active hemp fields and nearby fertilized areas. Factors most likely to aid escape include the distribution of viable plant material, for hemp that is seed, both accidental (e.g., equipment) or natural (e.g., wildlife), which may be more intensive in commercial-scale hemp seed and fiber production (Barney 2012). Commercial harvest goals for seed production are 1000-2000 kg/ha of dry seed from a field planted at 40-50 kg/ha live seed (Johnson 2019). It is possible for hemp to volunteer and disrupt subsequent crops from seed transported to a field or remaining after harvest. Translocation and establishment beyond planted areas was not observed in our study.

Like many weedy species, we found that hemp was likely to volunteer from seeds in agricultural soil conditions. We found that when seed dropped to disturbed and abandoned fields in South Florida, hemp produced volunteer plants, though in pulses with variable and declining quality. Volunteer plants established and reproduced in multiple seasons during a calendar year and were observed for up to two years. Characteristics of agricultural soil (disturbance and fertility) contributed to volunteer plant establishment and reproduction, though fertility was only a moderate factor in our analysis. Volunteers were tracked for multiple years in fields experiencing approved termination and simulated abandonment and in other nearby areas. No volunteer was observed outside the planted areas. Maximum seed production appeared to be from clusters of relatively tall plants, likely representing a more vigorous variety matching the latitude and tropical climate of South Florida.

By rule, the industry is expected to avoid hemp becoming feral as has occurred in other crops. In Florida, the castor bean (*Ricinus communis*) was introduced as a landscape plant. Over multiple generations, uncultivated offspring became adapted to local environments, while desirable traits diminished. Castor bean was listed as a Category II invasive species in 2001 by the Florida Invasive Species Plant Council (FISC) and became a prohibited agricultural crop (FISC 2023, Friedman et al. 2022). Early detection and eradication under FDACS non-native planting oversight involved *Mikania micrantha* and *Chrysopogon aciculatus* (pers comm, FDACS Division of Plant Industry). It is also important to maintain best practices and monitoring to minimize the escape of hemp from cultivation and reduce its effects on agricultural and natural areas. This can ensure that when grown as a crop, hemp can provide benefits without compromise to its economics or the environment.

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As with many field studies, we investigated only a single site with its associated soil type and climate using an opportunity to gather important observations on hemp invasion risk. Also, this study only notably evaluated disturbed and fallow agricultural conditions, and not directly more natural habitats, which may be of greater concern for invasiveness potential. There are nearby natural areas (<500m) that are repeatedly visited with no observation of hemp volunteers outside the agricultural site. Preliminary studies in central Florida looked at hemp volunteers in a grassy and forested natural area with low germination (<20%) and no recurring regeneration as well (Brym and Fankhauser 2021; pg 59). Our volunteer study plants resulted from up to 21 varieties of mixed suitability to our area, which implies variable seed production, whereas commercial hemp plantings would plant only varieties of proven suitability, and likely greater risk to volunteer. Follow-up trials with use of commercially-viable varieties across sites and years could further determine the areas and time durations for volunteer hemp plants that may threaten crops and nearby areas. An improved understanding of the invasive potential of hemp and other new crops can help with management decisions and prevent ecological and economic harm.

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Competing Interests

The authors declare none.

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Table 1. Select varieties and characteristics used for hemp grown at the University of Florida Tropical Research and Education Center (TREC), Homestead, Florida in 2019 and 2020 (Mylavarapu et al. 2020). These same varieties were used in planting trials of 2019 (previous studies) and the invasion risk test of 2020 (present study).

VARIETIES	USE	ORIGIN	SEXES
‘CFX-1’	Fiber	Canada	Monoecious
‘HAN-NE’	Grain	South China	Dioecious
‘HELENA’	Dual	Italy	Dioecious

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Table 2. Cumulative seed produced and plant heights (cm) for each variety and nutrient application at harvest for plants in the seed establishment experiment of 2020 (N1: nutrients added; N0: no nutrients added). Values are shown as average and standard error. Letters denote Tukey post-hoc grouping.

Feature, Grouping	Hemp variety			
	CFX-1	Han-NE	Helena	Combined
Seed/plant, all[1]	65 [a] ± 6.74	43.82 [a] ± 5.45	56.17 [a] ± 8.33	49.95 ± 6.32
Seed/plant, N1	-[2]	43.33 ± 5.79	76 ± 8.55	56.4 ± 7.12
Seed/plant, N0	65 ± 6.74	44.4 ± 5.68	16.5 ± 0.35	42.78 ± 5.47
Height, all	51 [a] ± 0.59	38.65 [ab] ± 1.79	48.92 [a] ± 2.42	43.39 ± 2.14
Height, N1	-	41 ± 1.32	56.44 ± 1.76	48.3 ± 1.99
Height, N0	51 ± 0.59	36.3 ± 2.16	32 ± 0.6	37.9 ± 1.97

[1] Combined nutrient treatments (N0 and N1)

[2] No plants produced.

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Table 3. ANOVA results for height as the response variable at harvest in the invasion risk test of 2020.

	Df	*Sums of Sqs*	*Mean Sq*	*P value*
Nutrients	1	1629	1629.16	0.001
Block	7	10621	1517.33	0.0001
Variety	2	4620	2310.17	0.0001
Residuals	337	67707	200.91	

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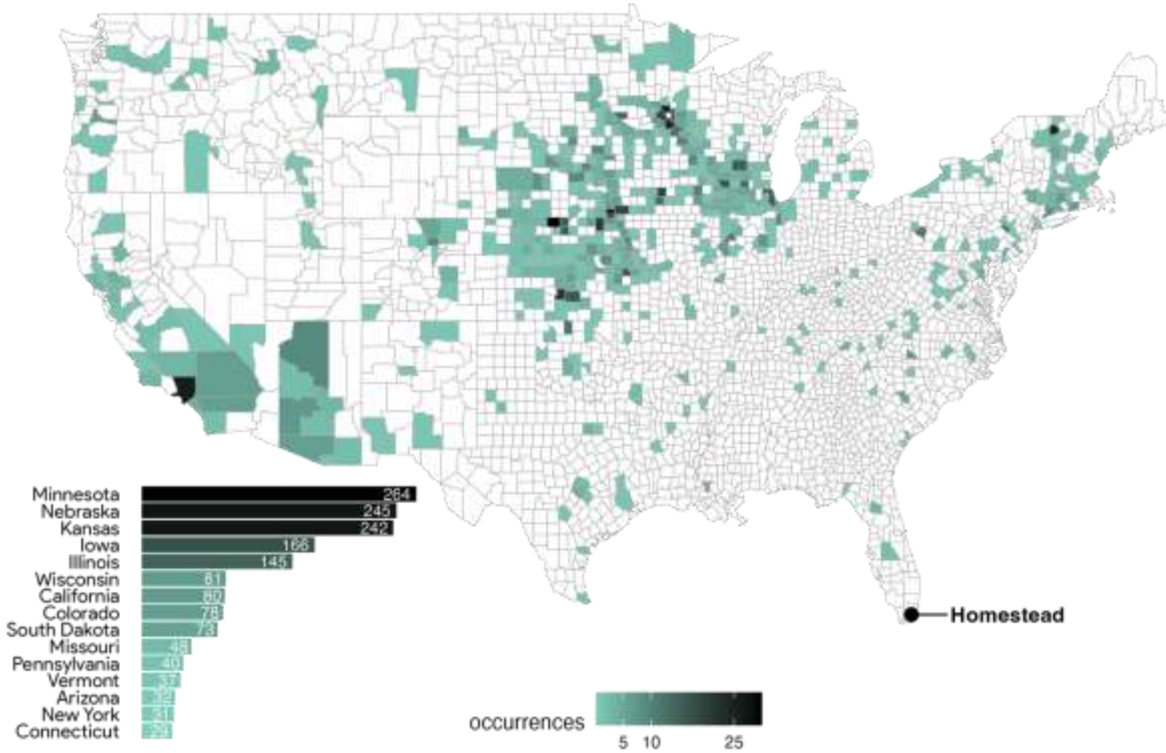

Figure. 1 Observations of feral *Cannabis sativa L.* per state and county in the continental United States (Data: GBIF 2024). Numbers within plot bars show sums of observations per state for the 15 states with the most observations. The bar for occurrences is a scale for the number of observations per county in the map.

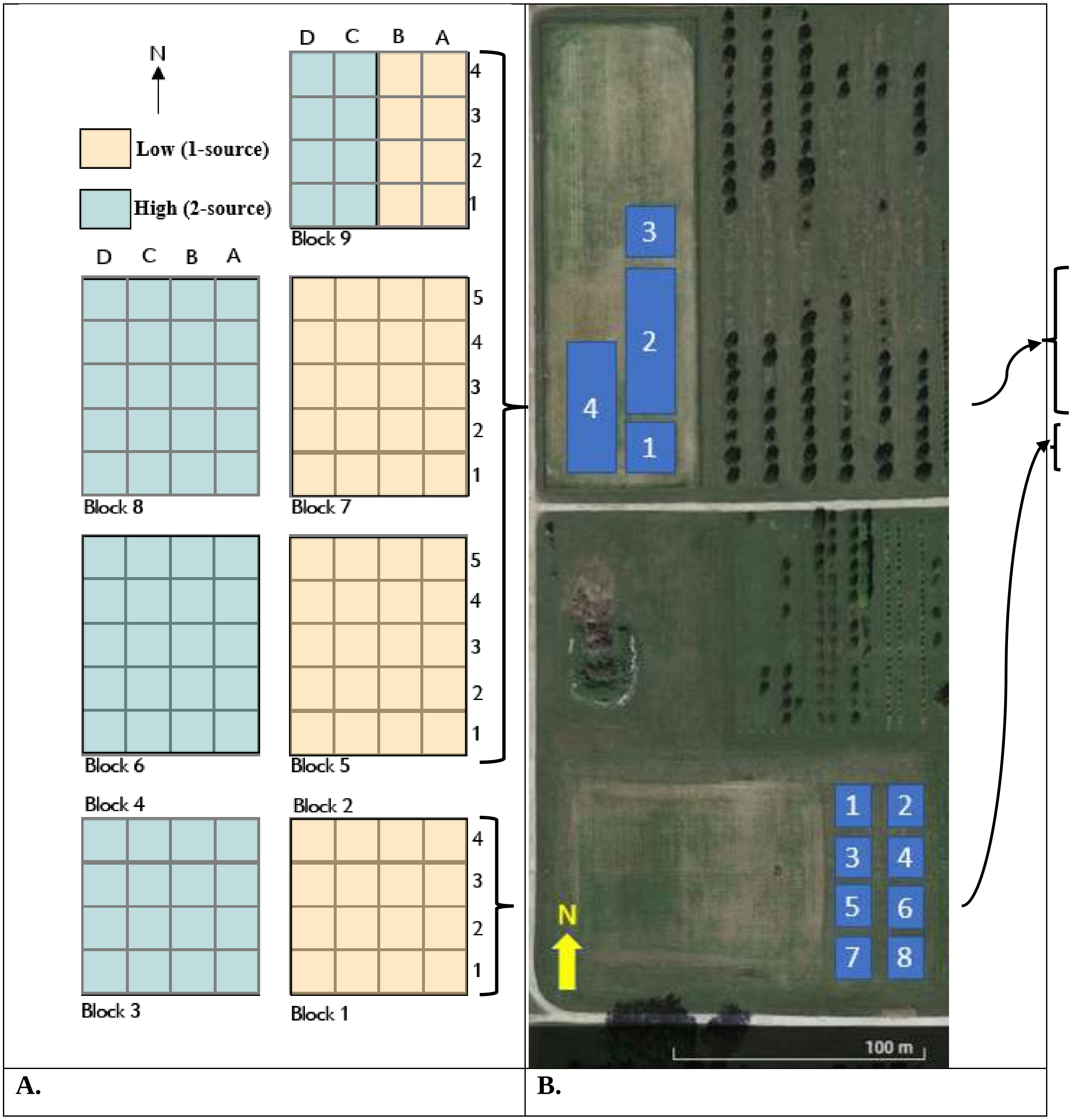

Figure 2. Layout and relationships between the four preceding 2019 trials and the 2020 field studies at the University of Florida Tropical Research and Education Center (TREC), Homestead, Florida. (**A)** The abandonment test conducted in 2020 and monitored through 2022. All the plots had propagule pressure resulting from seeds left in the field from the previous harvest in 2019. Pink highlighting (Treatment 1) indicates propagule pressure from only this source, while blue indicates additional propagule pressure was added as a second source (Treatment 2).A pilot project occurred in 2019 in blocks 1 – 4 with a variety trial in blocks 5-9. These collectively correspond to Blocks 1 and 2, respectively **(B** upper left**)**, with the accompanying Blocks 3 and 4 also used, but only in 2019. Our 2020 abandonment test occurred in the first two (#1-2) of these four trial sites which are numbered based on chronological order of planting date in 2019 (blocks #1-4, upper left). In the lower right are the eight blocks of the 2020 invasion risk test**.**

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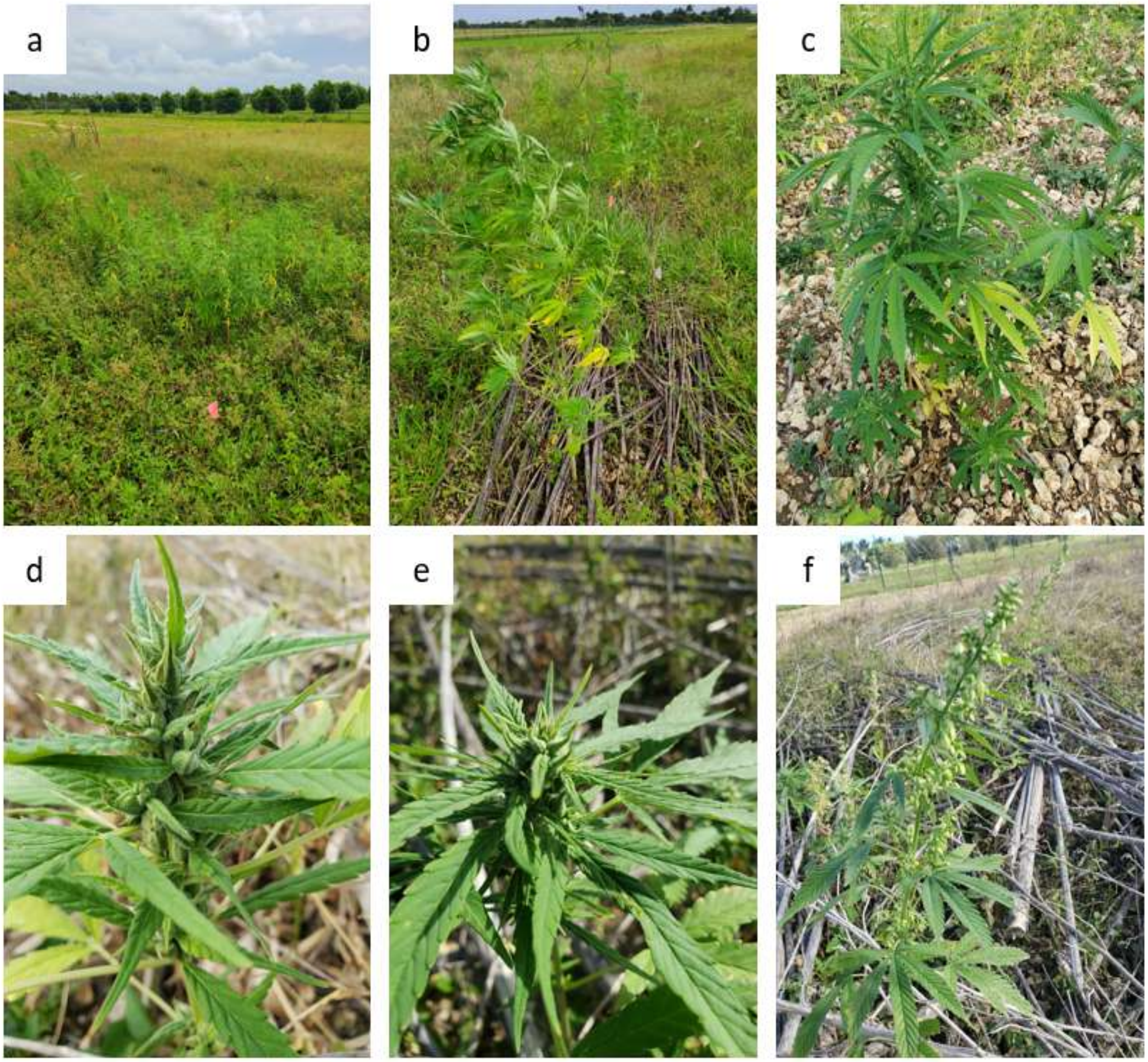

Figure 3. Volunteer hemp plants emerging (**a** and **b**), then flowering and setting seed (**c, d**, **e** and **f**) from post-harvest debris of the previous (2019) pilot project and variety trials at the University of Florida Tropical Research and Education Center (TREC), Homestead, Florida. Photos by Daniel Calzadilla 2020.

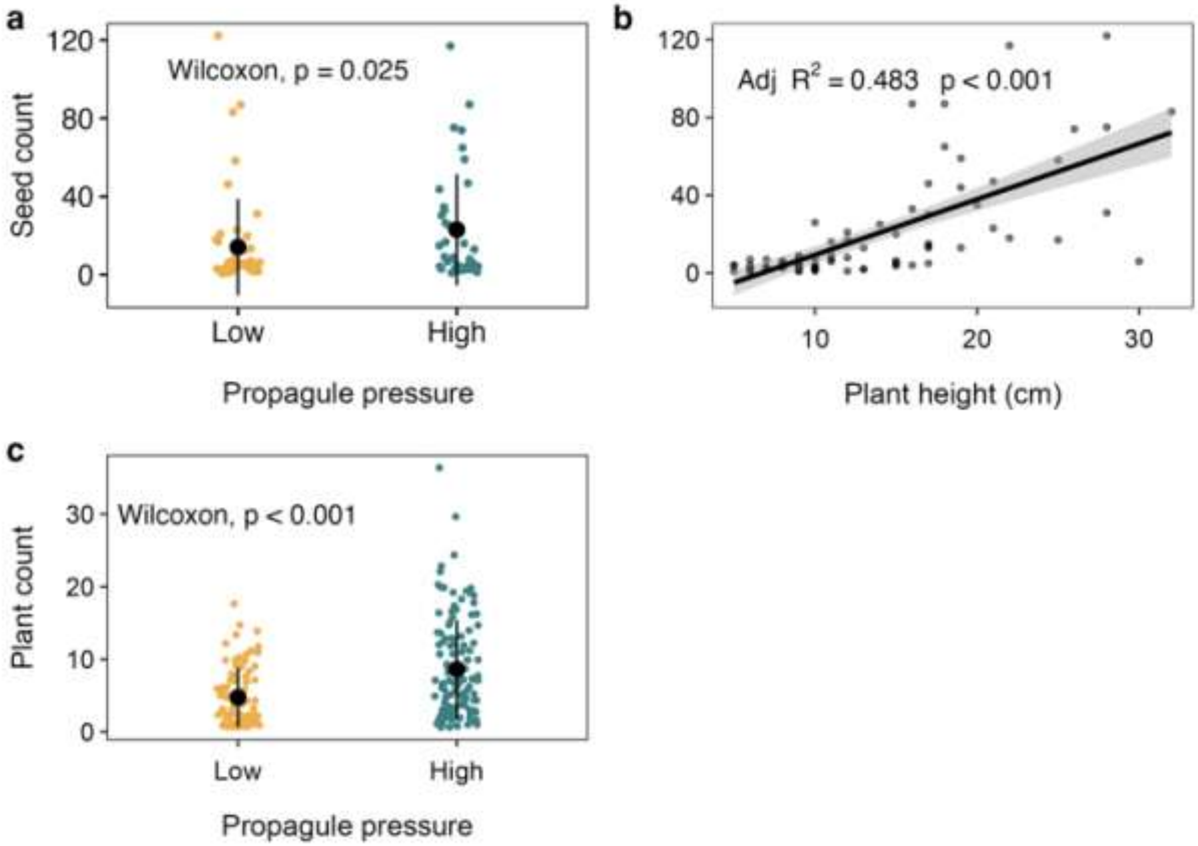

Figure 4. Volunteer population monitoring results from the initial monitoring period of Jan-Mar 2020. Analyses included the effects of (**a**) propagule pressure and (**b)** plant height on the number of volunteer hemp seeds collected and (**c**) plants counted in each propagule pressure treatment. Blue indicates high propagule pressure (2 seed applications) with gold denoting low pressure (1 application).

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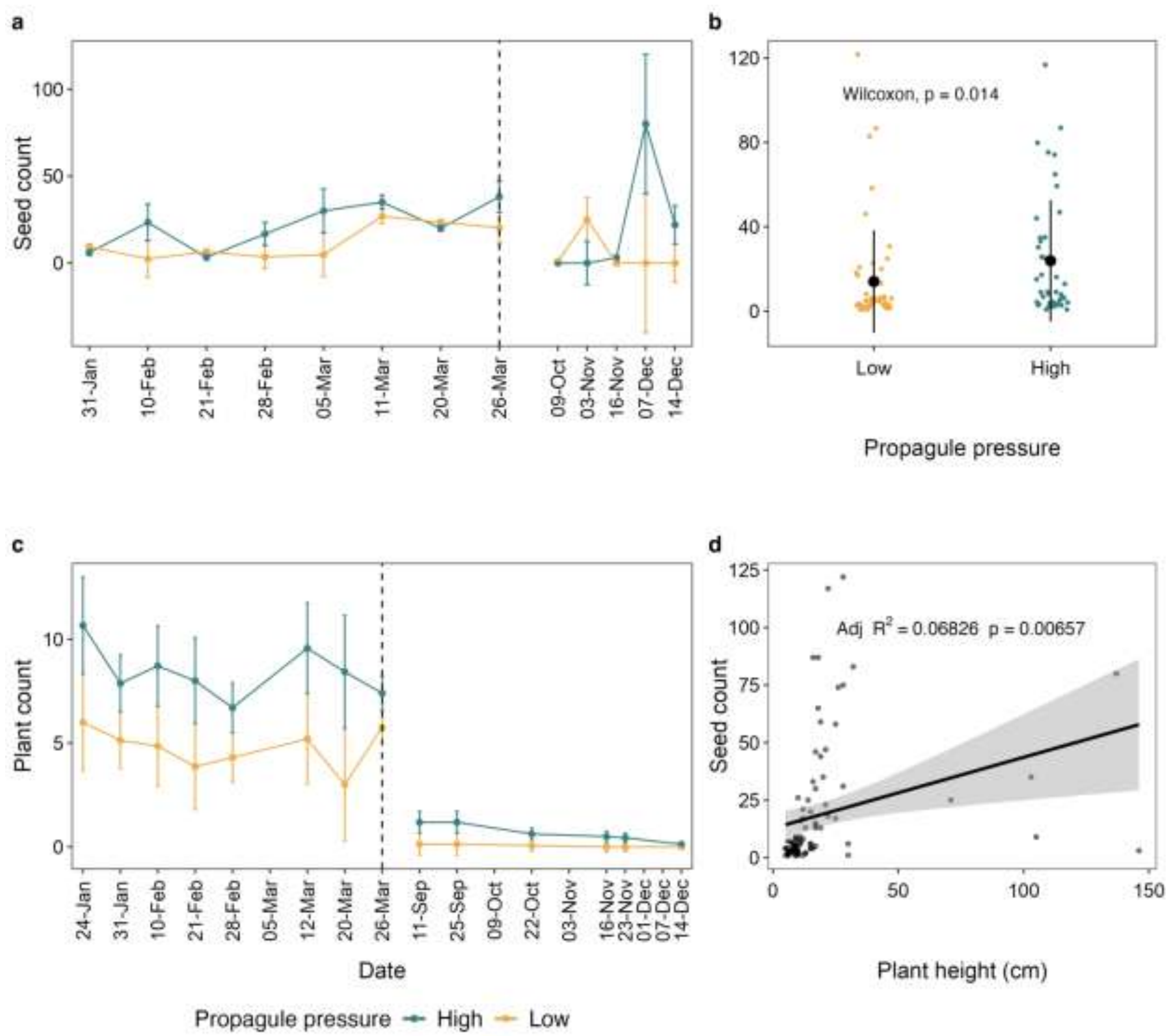

Figure 5. Volunteer population monitoring results for extended monitoring during Jan-Dec 2020. The initial phase of monitoring (Jan-Mar 2020, is left of the vertical dashed line, which shows the break in monitoring intensity. Data included mean numbers of (**a**) volunteer hemp seeds collected and (**c**) plants counted per observation date with dots and bars showing Mean ± SE for each observation date. Further analyses found the effects of (**b**) propagule pressure and (**d**) plant height on the number of volunteer hemp seeds collected. Blue indicates high propagule pressure (seed drop plus seed application), with gold showing low pressure (seed drop only).

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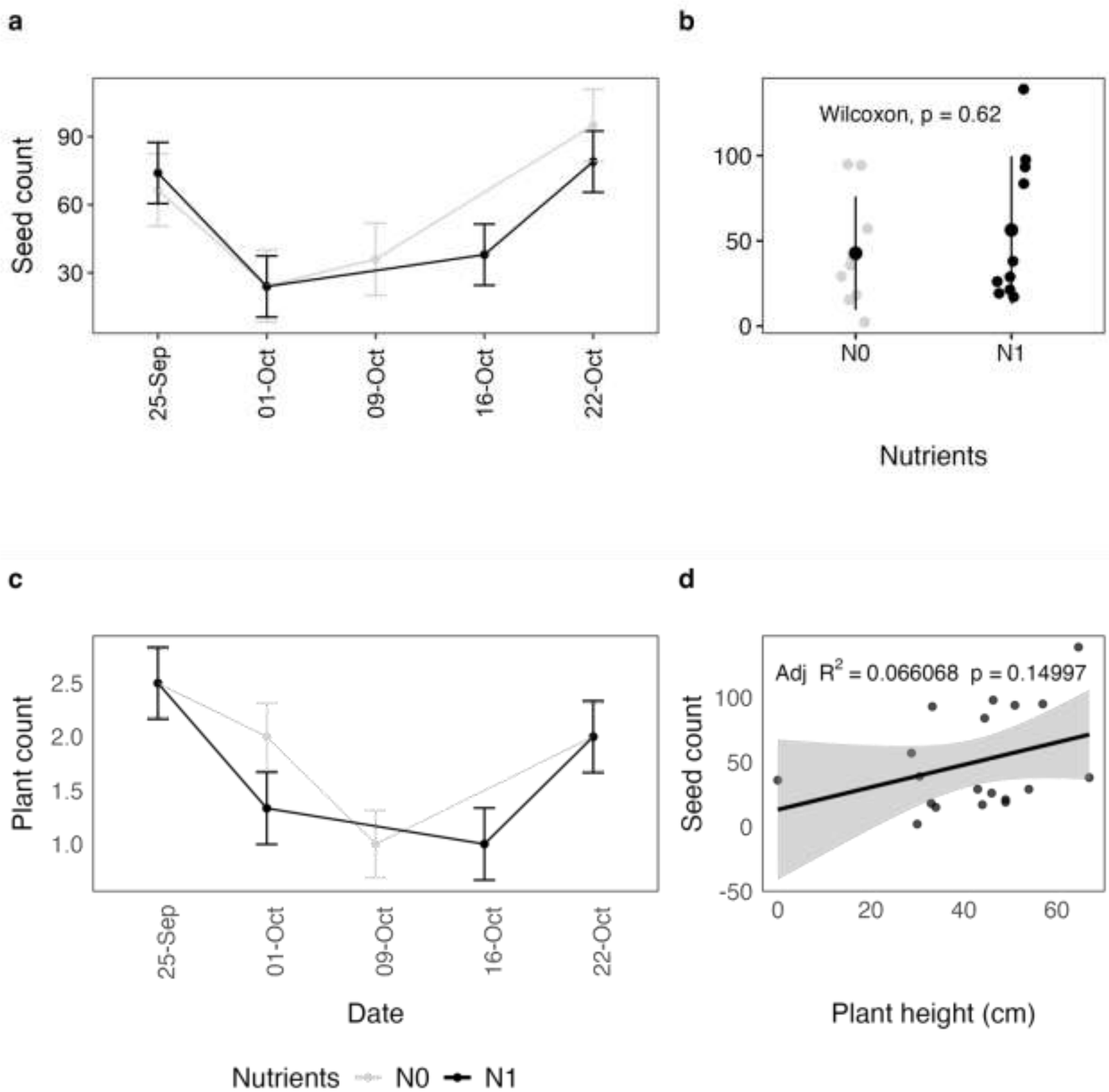

Figure 6. Seed establishment experimental results, 2020. Data included mean numbers of (**a**) volunteer hemp seeds collected and (**c**) plants counted with dots and bars showing Mean ± SE for each harvest date. Further analyses showed the effects of (**b**) nutrient treatment and (**d)** plant height on the numbers of volunteer hemp seeds collected. Dots represent the average value per treatment (a, c) and plot (b, d). Black indicates high nutrients and grey indicates low nutrients.

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