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Evaluation of Industrial Hemp Seed Treatments for Management of Damping-Off for Enhanced Stand Establishment

Hilary Mayton [1], Masoume Amirkhani [1,*], Michael Loos [1], Burton Johnson [2], John Fike [3], Chuck Johnson [4], Kevin Myers [5], Jennifer Starr [5], Gary C. Bergstrom [5] and Alan Taylor [1,*]

1 Cornell AgriTech, School of Integrative Plant Science, Horticulture Section, Cornell University, Geneva, NY 14456, USA; hsm1@cornell.edu (H.M.); mtl72@cornell.edu (M.L.)
2 Department of Plant Sciences, The College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, Fargo, ND 58108, USA; burton.johnson@ndsu.edu
3 School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061, USA; jfike@vt.edu
4 Southern Piedmont Agricultural Research and Extension Center, Virginia Tech, Blackstone, VA 23824, USA; spcdis@vt.edu
5 School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Cornell University, Ithaca, NY 14853, USA; klm25@cornell.edu (K.M.); jennifer.starr@iff.com (J.S.); gcb3@cornell.edu (G.C.B.)
* Correspondence: ma862@cornell.edu (M.A.); agt1@cornell.edu (A.T.)

Abstract: The purpose of this research was to collect efficacy data on biological, biochemical, and chemical fungicide seed treatments on hemp (*Cannabis sativa* L.) to mitigate damping-off and enhance field stand establishment. Seed treatments were evaluated in fields in New York (NY), North Dakota (ND), and Virginia (VA) and at two planting dates in each state in 2020. A single seed lot of a dual-purpose (fiber + grain) cultivar ('Anka') was treated using a laboratory-scale rotary pan coater. Five biological, two biochemical, and four chemical seed treatments were tested. A laboratory germination test revealed that seed treatments did not exhibit phytotoxicity when compared to the non-treated control. A laboratory bioassay with naturally infested soil was used to assess the preliminary activity of seed treatments for protection against damping-off. The biochemical seed treatment Ultim® (active ingredient; organic copper) performed as well as the chemical treatments Apron XL® + Maxim® 4FS and Mertect® 340F in preventing damping-off whereas the biological treatments did not differ from the non-treated control in terms of disease incidence. In all field tests, biological seed treatments did not improve plant stands compared to the non-treated control. Biochemical seed treatments Prudent 44® with Nutrol® (active ingredient; phosphite) and Ultim®, along with chemical seed treatments, had acceptable efficacy and improved stand establishment compared to the non-treated control across field locations. Based on efficacy results from laboratory and field trials, the copper seed treatment has potential for both conventional and organic hemp production.

Keywords: biopesticides; biological control; *Pythium*; *Cannabis sativa* L.; organic copper; phosphite

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1. Introduction

There is a renewed interest in the commercial production of hemp (*Cannabis sativa* L.) in the United States (US) following the passage of the 2018 US Farm Bill that removed the crop from Schedule I of the Controlled Substances Act [1,2]. *C. sativa* L. is most often associated with its production of psychoactive terpenophenolic cannabinoids, specifically, Δ-9-tetrahydrocannabiol (THC) [3,4]. However, industrial hemp with <0.3% THC is grown for fiber, grain, and hempseed oil or for use as a nutraceutical primarily for cannabidiol (CBD) production [2,3,5]. Hemp grown for grain and fiber is normally direct-seeded in the field, whereas cannabinoid hemp is often grown in a greenhouse or transplanted to the field [6]. Several recent publications reported numerous pest and disease problems associated with the production of hemp [7–9]. Damping-off, along with powdery mildew, *Botrytis* grey mold, and multiple leaf spot diseases, were all cited as impediments to

commercial hemp production [9–13]. In addition, seedlings started in the greenhouse often have poor root development that leads to increased susceptibility to pathogens and biotic stresses when transplanted into the field.

Damping-off is specifically identified as a major problem in both field and greenhouse production in the US and Canada [8,14–17]. The primary pathogens responsible for damping-off in several studies were identified as *Pythium* sp., *Fusarium* sp., and *Rhizoctonia* [8,14,16,17]. The US restrictions placed on hemp in the 1930s limited research and no pesticides were developed for disease control. Seed treatments are urgently needed for efficient early season pest management, including the control of damping-off caused by soil associated pathogens. Currently, pelleting, film coating, and encrusting are coating technologies used in the seed industry as delivery systems for plant protectants [18]. However, the hard, waxy surface of hemp seeds necessitates film coating formulations for uniform seed treatment application. Organically approved treatments would be ideal so that they could be used in both conventional and organic production systems. Significant advances were made in recent decades in the application of biopesticides as seed treatments that have resulted in increased efficacy and more consistent disease control [19].

Biopesticides comprise two main categories, namely microbial-based products that are either composed of microorganisms and the second category, which are biochemicals. Biochemical products include minerals, plant and microbial extracts, enzymes, and organic acids [20]. Several studies showed that biological seed treatments (BCA) that use *Trichoderma* and *Bacillus* species alone or in combination suppressed disease development and improved plant growth [21–23]. Corn (*Zea mays*) seed coated with *Trichoderma atroviride* had greater emergence and efficacy against two *Fusarium* pathogens in greenhouse and field studies, and *Trichoderma* applications also enhanced plant defense and germination when applied to cucumbers (*Cucumis sativus* L.) [24,25]. Zaim et al. [26] demonstrated that a combination of *B. subtilis* and *T. harzianum* applied as seed treatments on chickpea suppressed disease development caused by *F. oxysporum* f. sp. *ciceris* by 93% and enhanced plant growth. A combination of *B. subtilis* and *T. asperellum* applied as seed treatments on tomato were effective in controlling *Pythium aphanidermatum* [23]. Biological control of damping-off with a commercial formulation of *Clonostachys rosea* applied as a combination of seed treatment and soil drench was as effective as mefenoxam in reducing disease in seedlings of American ginseng (*Panax quinquefolius*) [27]. However, a commercial formulation of *Trichoderma harzianum* was ineffective in this same study.

Biochemical seed treatments may be derived from minerals and elements, including copper and phosphorus. Phosphites are widely studied in agriculture, and phosphites as an alternative for the management of plant disease were reviewed in [28,29]. Phosphites, also known as phosphonates, are derived from phosphorus acid (H_3PO_3) and are commonly combined with potassium, sodium, calcium, or ammonia. Phosphites exhibit systemic movement in the plant and move in both the xylem and phloem. Phosphites have demonstrated efficacy for a broad range of plant pathogens (oomycetes, fungi, bacteria, and nematodes) in studies conducted on specific crops and applied with different application technologies. Inorganic copper compounds were used for over a century as seed treatments for the control of plant pathogens, and this was reviewed by Leukel (1936) [30]. The early copper seed treatments were composed of copper sulfate, copper carbonate, or copper hydroxide and formulated as dusts. Because inorganic copper treatments could cause seed injury (phytotoxicity), they were largely replaced with synthetic fungicides [31]. The chemical synthetic seed treatment fungicides mefenoxam and fludioxonil are labeled on a wide range of crops [32,33]. Mefenoxam is effective against *Pythium*, while fludioxonil controls soil-borne fungi, including *Fusarium* and *Rhizoctonia*. The efficacy of these chemical seed treatments has not been reported on hemp.

Currently, there are neither registered organic controls nor chemical pesticides approved for use on hemp in the US. The purpose of this research was to evaluate a number of biological, biochemical, and chemical seed treatments for the management of damping-

off caused by several pathogens (*Pythium*, *Fusarium*, and *Rhizoctonia*) to enhance stand establishment.

2. Materials and Methods

2.1. Seed, Seed Treatments, and Laboratory Seed Treatment Testing

Seed of a single hemp seed lot (cv'Anka', a monoecious dual purpose-fiber and grain variety) was acquired from UniSeeds, Cobden, ON, Canada. The seed lot was first sized by seed width to remove small seeds (<7.5/64 inch, or <3 mm). Seeds were treated at Cornell AgriTech, Geneva, NY, USA, using a laboratory-scale rotary pan coater, R-6 (Universal Coating Systems, Independence, OR, USA). Seed treatments and active ingredients are listed in Table 1. The 20CU_2697LQ from ABM (now Agrauxine Corp.), Van Wert, OH, USA, contained *T. atroviride* at a concentration of 2.6×10^9 colony forming units (cfu)/mL. Amplitude™, *B. amyloliquefaciens* strain F727, containing 1.0×10^9 cfu/mL, was provided by Marrone Bio Innovations, Davis, CA, USA. Bio Seed contained five biologicals at 1.0×10^8 cfu for each species and was provided by Ag Biotech, Lakeville, NY, USA. Phyter from Endo Plant Health, Oakville, ON, Canada, consisted of *C. rosea* at 1.0×10^9 cfu/mL. Varnimo contained 1.0×10^9 *B. amyloliquefaciens* cfu/g, and KaPre Embrella, Prudent 44® and Nutrol® were provided by LidoChem Inc, Hazlet, NJ. Ultim®, a copper hydroxide formulation, was obtained from Germains, Gilroy, CA, USA. All chemical fungicides were acquired from Syngenta, Greensboro, NC, USA. EnVigor, an organic-based, multi-functional seed coating polymer from Agrauxine, was used as the seed treatment binder for all treatments except for the Ultim® seed treatment.

Table 1. Seed treatments were applied to the Anka hemp seed lot with a laboratory-scale rotary pan coater.

Treatment #	Product	Active Ingredient	Rate/100 g Seed
1	Control	-	-
2	20CU_2697LQ	*T. atroviride*	426 µL
3	Amplitude™	*B. amyloliquifaciens* strain F727	250 mg
4	Bio Seed™	*Paenibacillus azotofixans*, *B. megaterium*, *B. mucilaginosus*, *B. subtilis*, *T. harzianum*	400 mg
5	* Varnimo/KaPre Embrella	*B. amyloliquifaciens*	200 + 2400 mg
6	Phyter	*Clonostachys rosea*	50 mg
7	Ultim®	Copper hydroxide	0.05 mg Cu/seed
8	Prudent 44®/Nutrol®	Phosphite material + Monopotassium phosphate	640 mg + 80 mg
9	Apron XL®/Maxim® 4FS 1/2X	Mefenoxam + Fludioxonil	½ X rate
10	** Apron XL®/Maxim® 4FS 1X	Mefenoxam + Fludioxonil	1 X rate
11	Apron XL®/Maxim 4FS® 2X	Mefenoxam + Fludioxonil	2 X rate
12	*** Apron XL®/Maxim®4FS /Mertect® 340F 1X	Mefenoxam + Fludioxonil + Thiabendazole	1 X rate for all products

* KaPre Embrella contains monosaccharides, disaccharides and oligosaccharides and natural compounds for microbes. ** The 1X rate of Apron XL®/Maxim® 4FS is 23.2 mg, 6.3 mg, and 11.8 mg/100 g, respectively. *** The 1X rate of corresponding active ingredients of Mefenoxam/Fludioxonil/Thiabendazole is 7.5 mg, 2.5 mg, and 5.0 mg/100 g, respectively.

A preliminary roll-towel soil bioassay was conducted to test the germination and efficacy of the seed treatments. Field soil (Arkport fine sandy loam) was obtained from an East Ithaca, NY, field location and contained the damping-off pathogen *P. aphanidermatum* (Figure 1). The soil media used for the bioassay was composed of (by weight) 10% of the field soil thoroughly mixed with 90% sand. Twenty-five seeds were placed linearly, 6 cm

from the edge of the moistened towels. A straight-edge shield was placed 4 cm from the edge of the towel, allowing the seeds to be covered with 2 cm of soil, simulating in-furrow planting depth. Using 100 g of the prepared soil media and a #10 sieve, the soil was evenly shaken over the seeds and towel surface, then covered with a third towel and folded. Four replicates were prepared using a roll-towel protocol [34], and germination results were scored on days 4 and 7 with a final count on day 10 (DAP) (Figure 1).

Figure 1. Soil bioassay naturally infested with *Pythium aphanidermatum*. Ratings were recorded 4, 7 and 10 days after planting (DAP).

Germination test criteria were adopted from the Association of Official Seed Analysts (AOSA) manual [34], and germination was counted as a positive score when the seedling radical was at least 2mm in length. For final counts, seedlings were only considered viable when they met AOSA standards of healthy roots, hypocotyl, epicotyl, the presence of at least $\frac{1}{2}$ of cotyledon tissue, and no disease presence. Seedlings were scored as normal, abnormal, or dead. Seeds were germinated in a Percival germinator (Percival Scientific, Inc., model I36LL, Perry, IA, USA) set at 15/25 °C, night 10 h, day 14 h, with light provided during the day period. The alternating 15/25 °C was selected based on the average diurnal soil temperatures on June 1st in Geneva, NY, USA [35]. In addition, a rolled towel germination test was conducted without soil to assess seed treatment phytotoxicity. Seedlings were scored as normal, abnormal, or dead after 7 days, and the germination test was also conducted in the same Percival germinator at 15/25 °C, night 14 h, day 10 h, with light provided during the day period.

2.2. Field Trials

Trial locations, planting dates, and the dates of final seedling counts were recorded, and soil types are shown in Tables 2 and 3. All planting sites were tilled prior to planting. In NY, 100 seeds were sown per plot with four replications for both trials with a push planter. The first trial in NY was planted on 12 June. The soil was an Arkport fine sandy loam. The second trial in NY (Hudson/Collamer silt loam soil) was sown on 26 June. North Dakota trials were planted on 20 June and 29 July into fine and firm seedbeds with a Bearden/Perella silty clay loam soil. The field was conventionally prepared for a small-seeded crop. Seeding in ND was performed using a 6-row (spaced at 30 cm), 3-point mounted planter with double disk openers with twin-vee packer wheels. The center four-planter rows were sown with 100 seeds placed at a 20 mm depth with a row length of 3.05 m. The first site in VA (Dothan/Norfolk sandy loam soil), planted at the Southern Piedmont Agricultural Research and Extension Center, was established with a forage seeder on 10 April. Four replications were planted on each experimental plot, each plot containing two 6.1 m-long rows containing 100 seeds per row. The second trial was also seeded with four replications of 100 seeds in VA (Duffield/Ernst complex fine loam soil) and was planted with a walk-behind cone seeder adapted for two rows. The second site was seeded on 4 October following several spring plantings that failed due to flooding. The plots were tilled, and the soil was allowed to settle for a week before planting.

Table 2. Trial locations, planting and final stand count dates, and soil types at each location.

Trial Location	Planting Dates		Final Stand Counts (* DAP)		Soil Type	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
New York	12 June	26 June	21	21	Arkport fine sandy loam	Hudson/Collamer silt loam
North Dakota	20 June	29 July	27	27	Bearden/Perella silty clay loam	Bearden/Perella silty clay loam
Virginia	10 April	4 Oct	25	20	Dothan/Norfolk sandy loam	Duffield/Ernst complex fine loam

* DAP (days after planting).

Table 3. Trial GPS locations, and addresses.

Trial Location	GPS		Address	
	Trial 1	Trial 2	Trial 1	Trial 2
New York	42°26′27.7′′ N 76°28′15.0′′ W	42°27′2.268′′ N 76°26′52.342′′ W	East Ithaca, NY	Dryden, NY
North Dakota	47°00′11.30′′ N 97°06′41.95′′ W	47°00′11.30′′ N 97°06′41.95′′W	Casselton, ND	Casselton, ND
Virginia	37°05′34.86′′ N 77°57′52.27′′ W	37°13′0.365′′ N 80°27′45.761′′ W	Blackstone, VA	Blacksburg, VA

Stand counts were recorded starting at day 7 and continued every 5–7 days for 3–4 weeks at each location. Stand counts were based on the above ground emergence of the cotyledons and used to estimate the percentage emergence for each measurement event. In addition, plants were evaluated for disease incidence via visual inspection for signs and symptoms of pathogen development. After final counts, samples were destructively harvested at some locations and examined for root rot. Samples with signs of disease were evaluated for pathogens at each location.

2.3. Field Pathogen Diagnosis

Diseased seedlings from field trials in Ithaca, NY, were collected while conducting stand counts and taken to the Bergstrom laboratory located at Cornell University for identification. Samples were surface sterilized for 1 min each in 70% ethanol, 90% hypochlorite, and sterile distilled water. Tissue was then plated on potato dextrose agar (PDA) with the addition of streptomycin and neomycin and an oomycete selective agar (combination of pea and rye B agar) [36]. Plates were incubated at room temperature with a light cycle of 12 h of UV-light and 12 h of darkness for 3–5 days until colonies could be isolated. Once isolated into a pure culture, samples were identified morphologically and via sequencing of the ITS rDNA region. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen), followed by PCR amplification of the ITS rDNA region using the universal ITS primers ITS1 and ITS4 and the PCR method of White et al., 1990 [37]. The PCR fragments were purified using a Monarch PCR and DNA clean-up kit (New England Biolabs), and Sanger sequenced using Big Dye Terminator Cycle Sequencing and Clean-up Kits (Applied Biosystems, Inc., Waltham, MA, USA), followed by analysis on an ABI 3730XL DNA analyzer. The resulting sequence data were further processed using GeneStudio Sequence Analysis Software (GeneStudio, Inc., Suwanee, GA, USA), followed by sequence best match identification using NCBI BLAST [38].

Seedlings from North Dakota trials were carefully washed to remove soil that was attached to the roots. Entire plants were examined with a dissecting microscope (80× magnification) to observe general symptoms such as lack of roots, injuries, and discolored tissue. When a pathogen was suspected, tissues (roots and stems were cut lengthwise, leaves were cut into small pieces) were placed on temporary wet mounts and viewed on a compound microscope (400× magnification). Slides were examined for the presence of fungal structures (hyphae, spores) and bacterial streaming. Suspect arthropod-pest afflicted tissues were examined by an entomological diagnostician to confirm the cause. No disease was observed in trials located in Virginia.

2.4. Statistical Analysis

All seedling data from the trials were evaluated for normality, homoscedasticity, and goodness of fit, along with the generation of histograms of the distribution of residuals and normal probability plots, using JMP Pro Version 15 SAS Institute Inc., Cary, NC, USA, 1989–2021. All data (including a soil bioassay; Table 4) were subjected to arcsine square root transformation and evaluated for normality, homoscedasticity, and goodness of fit using the same procedures described above. Non-transformed percent emerged seedlings data had normal distributions in all trials across locations, and transformation did not improve normality or tests of goodness of fit. Therefore, non-transformed raw data were used to determine statistical differences in the percentage of emerged seedlings. Analysis of variance was assessed, and a comparison of means was conducted with Tukey's procedure using JMP Pro 15 at a significance level of $\alpha = 0.05$.

Table 4. Percent germination test results (Normal seedlings, Abnormal seedlings and Dead) and Soil Bioassay results for percent viable seedlings (4 Day, 7 Day, 10 Day).

		* Percent Germination Test			* Soil Bioassay		
#	**Treatment**	**Normal**	**Abnormal**	**Dead**	**4 Day**	**7 Day**	**10 Day**
1	Non-treated control	89	4	7	97 A	34 C	1 B
2	20CU_2697LQ	83	5	12	82 B	17 C	0 B
3	Amplitude	79	9	12	84 AB	19 C	8 B
4	Bio Seed	84	3	13	89 AB	21 C	0 B
5	Varnimo/KaPreEmbrella	86	5	9	85 AB	51 BC	7 B
6	Phyter	87	2	11	93 AB	31 C	0 B
7	Ultim	85	3	12	97 A	89 A	71 A
8	Prudent44 + Nutrol	79	5	16	83 AB	49 BC	8 B
9	Apron XL + Maxim 4FS 1/2X rate	84	8	8	90 AB	80 A	65 A
10	Apron XL + Maxim 4FS 1X rate	85	5	10	94 AB	85 A	67 A
11	Apron XL + Maxim 4FS 2X rate	81	9	10	83 AB	79 A	68 A
12	Apron XL + Maxim 4FS + Mertect 340F 1X	81	8	11	87 AB	71 AB	60 A
	$p \leq$ value	0.2 NS	0.08 NS	0.5 NS	0.0023 **	<0.0001 **	<0.0001 **

* Arcsine data transformation was performed prior to ANOVA (Tukey HSD). NS—Not significant at 0.05. Mean values with uncommon letters are statistically different. ** Significant at 0.01.

3. Results

3.1. Seed, Seed Treatments, and Laboratory Seed Treatment Testing

Normal, abnormal, and dead seedlings were counted in roll towel germination tests. No differences in any germination parameters were observed for biological, biochemical, and chemical seed treatments (#9–12, Tables 1, 4 and 5) when compared to the non-treated control (Table 4). Results from the soil bioassay showed that only Ultim, an organic copper treatment and chemical seed treatments (Apron XL + Maxim 4FS and in combination with Mertect 340 F [rates presented in Tables 1, 4 and 5]) reduced damping-off compared to the non-treated control and other treatments after 7 and 10 days. Varnimo and Prudent + Nutrol showed some efficacy after 7 days; however, after 10 days, they were not significantly better than the non-treated control.

Table 5. Final stand counts (% emerged seedlings/plants) in two trials of hemp seed treatments across three locations in 2020.

	Location	New York		North Dakota		Virginia	
		Trial		Trial		Trial	
#	Treatment	1 [w]	2 [w]	1 [x]	2 [x]	1 [y]	2 [z]
1	Non-treated control	33 C	27	37 CD	28 DE	14 CD	16
2	20CU_2697LQ	34 BC	32	36 CD	32 C-E	17 A-D	9
3	Amplitude	29 C	28	36 CDE	28 DE	15 BCD	13
4	Bioseed	31 C	30	33 D	34 B-D	12 D	10
5	Varnimo/KaPre Embrella	28 C	21	30 D	29 DE	16 BCD	22
6	Phyter	33 BC	24	40 C	26 E	14 CD	17
7	Ultim	42 A	35	49 B	42 A	21 A	28
8	Prudent 44 + Nutrol	41AB	29	38 C	27 DE	21 AB	17
9	Apron XL + Maxim 4FS 1/2X rate	32 C	22	58 A	43 A	20 ABC	17
10	Apron XL + Maxim4FS 1X rate	46 A	33	58 AB	40 AB	10 D	25
11	Apron XL + Maxim 4FS 2X rate	41 AB	42	56 AB	41 A	17 A-D	22
12	Apron XL + Maxim 4FS + Mertect 340F 1X rate	30 C	28	55 AB	36 ABC	15 BCD	20
	$p \leq$ value	0.0004 **	0.09 [NS]	0.001 **	0.0001 **	0.05 *	0.12 [NS]

*, ** Significant at 0.05, and 0.01. [NS]—Not significant at 0.05. Mean values with uncommon letters are statistically different. Final Count Days After Planting: [w] = 24 days, [x] = 27 days, [y] = 35 days, and [z] = 20 days.

3.2. Field Trials

Conditions were dry with above average air temperatures for the first trial in NY planted on 12 June. The second trial in NY (Hudson/Collamer silt loam soil) was sown on 26 June and was again planted under dry conditions, followed by a hot and dry period. Soil temperatures were greater than 16 and 21 °C for the North Dakota trials for the two seeding dates, respectively, and rainfall 7 days post-planting was 9.6 mm and 0.0 mm for seeding dates 20 June and 29 July, respectively. Although rainfall was minimal 7 days post-planting, soil water status was adequate for seed germination for both seeding dates due to rainfall occurring 7 days before planting.

Weather conditions were cool and damp at planting for the first site in VA, followed by >5 cm of rain 24–30 h after planting. For the second trial in VA (Duffield/Ernst complex fine loam soil), soil conditions were favorable, but seeding depth was inconsistent, and there was little rainfall after planting.

Overall, the % of final emerged seedling counts measured for the two trials established at different locations and planting dates (Tables 3–5) ranged from 9–58%. The highest percentage of final emerged seedling counts were observed in ND and the lowest in VA (Table 5). Trials in all three states had consistent seedling counts for both planting dates. New York trials ranged from 28–46% and 21–41% in the first and second planting dates, while ND ranged from 30–58% and 26–41%, respectively. Virginia with the lowest stand counts ranged from 10–21% in the first trial and 9–25% in the second planting.

In five of the six seed treatment trials, most chemical seed treatments (#9–12, Tables 1 and 5) generally had higher stand counts than the biological seed treatments (#2–6, Tables 1 and 5). No differences were observed among seed treatments in the second trial conducted in VA. Apron XL/Maxim 4FS applied at the 1X and 2X rates, and the biopesticides phosphite and Ultim seed treatments had greater stand counts than the non-treated controls at E. Ithaca (Table 5). At the second location in NY, only Apron XL + Maxim 4FS 2X rate had better stands than the non-treated control (Table 5). The Ultim seed treatment (#7, Table 5) was

consistent across all locations and in most trials performed as well as the chemical seed treatments (#9–12, Table 5).

3.3. Pathogen Diagnosis

Pathogens and/or diseased plants were observed and diagnosed in trials located in NY and ND; however, no samples were collected from either VA trial. For trials in NY, species of the damping-off pathogens *Pythium*, *Fusarium*, and *Rhizoctonia* were detected in plants sampled at East Ithaca (Table 6 and Figure 2). Due to dry conditions, no observations of diseased roots and/or seedlings were obtained in the second NY trial. *Rhizoctonia* was positively identified to species from samples treated with Amplitude in the first trial in ND and also from the control, Apron XL Max $\frac{1}{2}$X rate, and Apron Max XL 2X rates in the second trial. *Ulocladium* and *Fusarium* were found in the Bio Seed treatment and Apron Max XL $\frac{1}{2}$X rate plots in trial 1 in ND (Table 7).

Table 6. Sample and positive identification of pathogens present on hemp seedlings in Ithaca, NY.

Seed Treatment	*Pythium* Species	*Fusarium* Species	*Rhizoctonia* Species	*Mucor* Species	*Penicillium* Species	*Trichoderma* Species
Control	x [5]	x [1]	x [4]	x		x
Amplitude (A) *	x [5]	x [1]		x		
Amplitude (B) *	x [5]	x [1] x [2]	x [4]	x		x
Apron XL®/Maxim® 4FS 1/2X		x [1] x [2] x [3]	x [4]	x		
Bio Seed	x [5]	x [1] x [2] x [3]	x [4]	x		
Varnimo/KaPre Embrella (A) *		x [1] x [2]			x	
Varnimo/ KaPreEmbrella (B) *		x [1]	x [4]			

[1] *Fusarium equiseti* [2] *Fusarium solani* [3] *Fusarium oxysporum* [4] *Rhizoctonia solani* [5] *Pythium aphanidermatum* * Letters (A) and (B) indicate two separate samples were taken from the field plot I-3 (replicate I treatment 3).

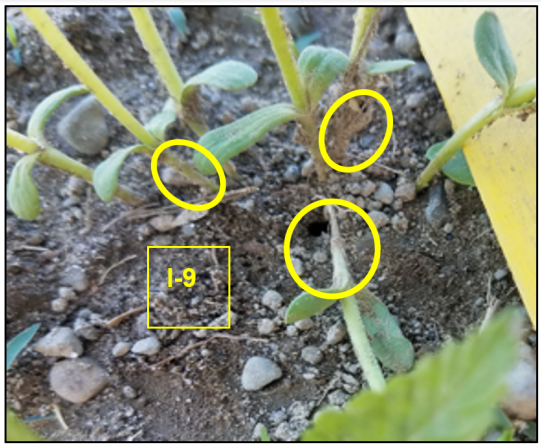

Figure 2. Pathogens were observed on seedlings from treatments 1, 3, 4 and 5 and 9 (Tables 1 and 6). Damping-off pathogens were identified as *P. aphanidermatum*, *F. equiseti*, *F. solani*, *F. oxysporum*, *R. solani*, *Mucor*, and *Trichoderma* species (Table 6) and the presence of bacterial species was also identified on hemp seedlings from trial 1 in NY. Pathogen tests were conducted in the Bergstrom lab, Cornell University.

Table 7. Sample and positive identification of pathogens present on hemp seedlings in Prosper, ND.

	Emergence Count Date	
Seed Treatment	**6 July**	**19 August**
Control	-	*Rhizoctonia*
20CU_2697LQ	-	-

Table 7. *Cont.*

	Emergence Count Date	
Seed Treatment	**6 July**	**19 August**
Amplitude	*Rhizoctonia*	-
Bio Seed	*Ulocladium*	-
Varnimo/KaPre Embrella	-	-
Phyter	-	-
Ultim	-	-
Prudent44/Nutrol	-	-
Apron XL/Maxim 4FS 1/2X	*Fusarium*	*Rhizoctonia*
Apron XL/Maxim 4FS 1X	-	-
Apron XL/Maxim 4FS 2X	-	*Rhizoctonia*
Apron XL/Maxim 4FS/Mertect 340F	-	-

Emergence count dates July 6 and August 19 correspond with planting dates June 20 and July 29, respectively. (-) indicates no pathogens detected. Pathogen tests were conducted at the North Dakota State University Plant Diagnostic Lab.

4. Discussion

High quality seed is the foundation for optimal stand establishment and the successful production of any crop. Moreover, seed quality is especially important for hemp as a new commercial crop with seed produced by a developing hemp seed industry. Currently, the seed of most fiber and grain varieties are imported into the US, and seed lots may be of low quality. Research at Cornell AgriTech's Seed Science and Technology program tested germination of 23 commercial hemp seed lots, and germination ranged from 29 to 94% in 2018 [39]. The minimum AOSCA standard for hemp is 80% germination [40], and only 13 of 23 lots tested in 2018 had greater than 80% germination. The germination of a hemp seed lot can be improved by post-harvest seed conditioning [41]. Small-sized seeds of three hemp varieties had a lower percentage of germination and produced seedlings with lower dry weight than larger sized seeds [42]. Thus, removing the small-sized seeds effectively upgraded seed lot quality. In this study, a commercial seed lot of 'Anka' was used, and approximately 10% of the seed lot was removed, resulting in the non-treated control with 89% germination (Table 1).

There are several important factors and conditions needed for the efficacy of biological control agents (BCA), starting with proper formulation and seed coating application technology [43,44]. The BCA used in this investigation were from commercial sources, so our assumption was that all the BCA were properly formulated. Envigor, a commercial seed treatment binder, was tested in preliminary experiments and the CFU (colony forming units) recovered from hemp seeds treated with a commercial mixture of *Trichoderma* spp. or *Bacillus amyloliquefaciens* remained high. Therefore, the lack of efficacy was not attributed to BCA formulation or seed coating technology. The environment in the soil bioassay created severe disease pressure resulting in <10% seedling survival after 10 days (Table 4). There are several mechanisms responsible for disease management in BCA, including mycoparasitism [45]. The biocontrol organism may need to achieve a critical population and produce its antifungal metabolites on roots in advance of pathogen growth in order to provide protection to the plant or enhance plant defense. In addition, hemp seedlings may be extremely susceptible to soil-borne pathogens, rendering these agents difficult to control. Further investigation is warranted to explore BCA efficacy and mechanisms in relation to damping-off on hemp seedlings.

Arguably, biochemical seed treatments have greater utility and flexibility than BCA seed treatments as they do not contain living organisms that must remain viable during the seed treatment process and post-treatment storage. Therefore, biochemical seed treatment

preparation, application, and storage are less complicated than BCA and thus more similar to the application of chemical seed treatments [18]. A phosphite seed treatment, Prudent 44 in combination with Nutrol, was included in this study (Table 1). Prudent 44 contains 57% by wt. urea phosphite and 15% by wt. ammonium phosphite [46]. Nutrol is monopotassium phosphate and is registered with the US EPA (Environmental Protection Agency), and Nutrol, when combined with Prudent, has expanded efficacy [47]. Since phosphites do not exist in nature, formulations containing phosphites are not approved for organic labelling. Prudent 44 with Nutrol provided some level of protection in the soil bioassay (Table 4) and two (Table 5-Trial 1 of New York and Virginia) of the six field tests. A commercial phosphite formulation (AG3) was applied as a seed soak, controlled damping-off caused by *Pythium* spp. in *Cucumis sativus* [48]. Phosphite applied as a seed treatment to corn reduced the mycelial growth of two *Fusarium* species [49].

Collectively, phosphites demonstrated potential as a hemp seed treatment, although efficacy was generally less than copper or the chemical fungicide seed treatments. Ultim treatment was performed as well as the chemical treatments at all locations and was the only treatment that was not significantly different from the chemical treatments in the soil bioassay (Tables 4 and 5). Soil type and planting conditions significantly influenced hemp stand establishment and growth; however, chemical seed treatments and Ultim were able to improve overall stand establishment at most locations. Currently, the registrant for the chemical seed treatments was not supportive of registering their materials for commercial use on hemp. The active component in Ultim is copper hydroxide, a patented formulation that is approved for organic use [50]. Collectively, the organic copper seed treatment has potential for both conventional and organic hemp production. Moreover, results from this study provide the foundation for seed treatment research on *Cannabis sativa* grown for other uses. For example, Ultim, the organic copper treatment evaluated in this study, and other organic copper seed treatments were found to be effective in controlling damping-off in the soil bioassay on a CBD (cannabidiol) variety of hemp [51].

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