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Comparison of Thermal Seed Treatments to Control Snow Mold in Wheat and Loose Smut of Barley

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Due to increasing demands to reduce chemical plant protection products, including prophylactic chemical seed treatments, there is a renewed interest in thermal seed treatments for cereal crops. We carried out contemporary evaluations of various alternative seed treatments for economically relevant cereal diseases in Switzerland. Thermal seed treatments were evaluated for effectiveness against two seed-borne diseases, snow mold (*Microdochium* spp.) and loose smut of barley (*Ustilago nuda*), commonly found in Swiss cereal production. Field trials testing seed treatments against *Microdochium* spp., including *M. majus* and *M. nivale*, on wheat were conducted across four growing seasons from 2016/17 to 2019/20 and against *U. nuda* on barley across three growing seasons from 2016/17 to 2018/19. The foci of these trials were primarily on thermal seed treatments, including steam, hot air, and warm water. Additionally, a Cerall® treatment, based on the microorganism *Pseudomonas chlororaphis* strain MA 342, was included in two of the trials focusing on *Microdochium* spp. Steam, warm water, and hot air showed high efficacy against *Microdochium* spp., while Cerall® showed no disease reduction. In the *Microdochium* spp. 2018/19 trial, a combination of poor field conditions, low quality seed, and high disease pressure reduced seed germination. The 2019/20 *Microdochium* spp. field trial, which occurred during less challenging field conditions than those in 2018/19 and included the same seed lot from 2018/19 and a less diseased second lot, showed an improved efficacy of the steam treatments. The warm water treatments were found to be the only effective thermal treatment against *U. nuda*. Our results demonstrate that the steam treatments more readily affected germination rate in a highly diseased seed lot, while warm water treatments showed limited damage to the seed. Warm water was found to be the most consistently effective thermal treatment against both diseases, and constraints in implementing such a treatment are discussed. If the steam treatment parameters are correctly set to minimize damage to the plant, it offers effective protection against some seed-borne diseases. Overall, the results from this study give more information about effectiveness of alternative seed treatments under various field conditions.

Keywords: alternative seed treatments, seed-borne diseases, *Ustilago nuda*, loose smut, *Microdochium* spp., snow mold, organic, cereal

INTRODUCTION

In conventional agriculture, preventive chemical-synthetic seed dressing is common practice for field crops. In Australia, the E.U. and the U.S., cereals account for some of the largest volumes of fungicide active ingredients applied as seed treatments (Lamichhane et al., 2020). Increasing interest in planting non-treated seed outside of organic grain cultivation may lead to a reduction of prophylactic seed treatments in cereals in integrated production (Lamichhane, 2020). Concerns about human and ecosystem health as well as the rise of fungal resistance to fungicides have led to increased societal pressure to reduce chemical pesticides (Nicolopoulou-Stamati et al., 2016; Berger et al., 2017). Several European countries have introduced national action plans since 2011 that mandate the reduction of pesticides (Directive 2009/128/EC), encouraging a reduction of preventative seed dressings. Similar to national action plans from several European countries (Barzman and Dachbrodt-Saaydeh, 2011; Möhring et al., 2020), a Swiss national action plan launched in 2017 aims to reduce the risk of plant protection products by 50% (Bundesrat, 2017).

Due to shifting public sentiment and the passage of national mandates, finding alternative seed treatments has gained more urgency. Synthetic chemical seed dressings are effective means to control seed-borne cereal diseases, which has made it challenging to adopt alternative methods. A few alternative seed treatments to combat seed-borne diseases are already available on the market for organic farming, including Cerall® and Cedomon® (Widén and Annas, 2004), which are both composed of the microorganism *Pseudomonas chlororaphis* strain MA 342 for wheat and barley, respectively. Additionally, Tillecur®, which is composed of mustard flour, has shown effectiveness against *Tilletia caries*, causing common bunt in wheat (Winter et al., 2001). However, these seed treatments can exhibit varying efficacies (Tinivella et al., 2009; Krebs et al., 2011), and Tillecur® currently is no longer registered in Switzerland. In addition to alternative treatments composed of microorganisms or plant products, thermal treatments have reemerged as an alternative possibility to existing synthetic chemical treatments (Winter et al., 1997; Forsberg et al., 2005; Koch et al., 2010).

Thermal treatment methods, such as the use of steam or warm water, are effective because pathogens are often more sensitive to elevated temperatures than the plant host (Baker, 1962). The temperature range and duration (i.e., treatment window) for controlling pathogens without damaging the seed embryo depends mainly on the seed's heat tolerance which is dependent on its physiological condition (Forsberg, 2004). Factors that affect the seed quality include age, handling history (i.e., possible exposure to mechanical damage from harvest or storage), and conditions during development or storage. A number of different thermal treatment methods have been described, including water baths and aerated steam with different derivations for various plant parts such as seeds, trees, cuttings, cut flowers, and sprouts (Grondeau et al., 1994). The use of heat to treat pathogenic organisms in wheat and barley was first reported over 100 years ago (Jensen, 1888). Since then, the effectiveness of warm water on cereal diseases has been documented to work against numerous diseases (Winter et al., 1997, 1998a). Despite their earlier documented efficacy, thermal methods have not been widely utilized on cereal seed due to the availability of inexpensive and efficient synthetic chemical treatments and low value of field crops (Lamichhane et al., 2020). As more pressure to reduce chemical synthetic seed treatments has grown, the reevaluation of thermal and alternative seed treatment methods is warranted.

In Switzerland, the main problematic seed-borne diseases in cereals include loose smut of barley (*Ustilago nuda*) (Hebeisen, unpublished data), snow mold (*Microdochium* spp.), and common [*T. caries* (DC.)] and dwarf bunt (*Tilletia controversa* Kühn) of wheat (Bänziger et al., 2012). The pathogens *M. nivale* (formerly *M. nivale* var. *nivale*) and *M. majus* (formerly *M. nivale* var. *majus*) are the causative agents of seedling blight, particularly under cool temperatures and sufficient snowfall, and can co-exist with each other (Nielsen et al., 2013). Using field and laboratory trials, various seed treatment methods, including a warm water and steam treatments as well as a biological control product were tested against a disease that is mostly present in non-embryo seed tissue (*Microdochium* spp.) and a disease located within the seed embryo (*U. nuda*). These experimental results help to inform which alternative seed treatments are appropriate to use against diseases that have different characteristics and locations within cereal seeds, and challenges associated with the effective treatments' implementation are discussed.

MATERIALS AND METHODS

Seed

Winter wheat (*Triticum aestivum* L. subsp. *aestivum*) seed naturally infected with *Microdochium* spp. and winter barley (*Hordeum vulgare* L. subsp. *vulgare*) seed naturally infected with *U. nuda* were used for the laboratory investigations and field trials (**Table 1**). For the 2016/17 *Microdochium* spp. trials, the Wiwa variety was used, whereas the Nara variety was used during all subsequent *Microdochium* spp. trials (2017/18, 2018/19, and 2019/20). The Runal variety was also included in the 2019/20 trial. All varieties are on the Swiss list of recommended varieties (Courvoisier et al., 2016) and were chosen due to the availability of naturally infected seed. These varieties were self-propagated at Agroscope and infection had been observed in the field during propagation. The same seed lots were used in the laboratory tests and field trials. For *U. nuda* trials, the variety, Cassiopee, was used for the 2016/17 trial and the variety, Caravan, was used for the 2018/19 trial. At the time of the study, Caravan was on the Swiss list of recommended cereal varieties, and Cassioppee has been listed in the European Commission's common varieties (European Commission, 2012).

Molecular Identification of *Microdochium* spp. in Seed

Because colonies of *M. majus* and *M. nivale* are difficult to visually distinguish from each other, we used species-specific PCR primers to determine if the seed used in this study was

TABLE 1 | Field conditions for *Microdochium* spp. on winter wheat and *Ustilago nuda* on barley field trials in growing seasons from 2016/17 to 2019/20.

Growing season	2016/17	2017/18	2018/19	2019/20
***Microdochium* spp.**				
Crop variety	Wiwa 2016	Nara 2017	Nara 2017	Nara 2017 and Runal 2019
Sowing date	24 October 2016	01 November 2017	19 November 2018	14 November 2019
Harvest date	18 July 2017	31 July 2018	22 July 2019	24 July 2020
Soil characteristics	Loam, clay loam, sandy loam	Sandy loam, clay	Sandy loam	Loam, clay loam, loam silt
Disease assessment	14 February 2017	30 January 2018	27 February 2019	24 February 2020
Precrop	Potatoes	Potatoes	Quinoa	Potatoes
Sowing rate	350 seeds/m^2	350 seeds/m^2	350 seeds/m^2	350 seeds/m^2
Row spacing	12.5 cm	15.5 cm	15.5 cm	17.5 cm
Ustilago nuda				
Crop variety	Cassiopee 2016	Caravan 2015	Caravan 2018	
Sowing date	4 October 2016	4 September 2017	25 October 2018	
Harvest date	26 June 2017	22 June 2018	3 July 2019	
Soil characteristics	Loam, clay loam, loam silt	Loam, clay loam, loam silt	Loam, clay loam, loam silt	
Disease assessment	15 May 2017	14 May 2018	14 May 2019	
Precrop	Canola	Canola	Canola	
Sowing rate	350 seeds/m^2	350 seeds/m^2	320 seeds/m^2	
Row spacing	15.5 cm	15.5 cm	17.5 cm	

infected with *M. majus*, *M. nivale*, or a mixture of the two species. Samples of 100 g of each seed lot used in the field and laboratory trials were milled into flour. DNA was isolated from a 100 mg subsample of the milled flour using the NucleoSpin Plant II kit for DNA from plants (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The primers used for the species-specific PCRs included distinct forward primers for each species: *M. nivale* (EFNiv/F: 5′-GTT CCC CTG TCT GAC TGT TGT-3′) and *M. majus* (EFMaj/F: 5′-CCC CTT CTC CCT ATC GC-3′) and a shared reverse primer (EFMic: 5′-GTC TCG ATG GAG TCG ATG G-3′), which have been previously published (Glynn et al., 2005). PCRs were performed in 20 uL volumes and included 50 ng of template DNA, 300 nM of each primer, 10 μL GoTaq® G2 Green Master Mix (Promega). PCR cycling conditions consisted of an initial denaturation of 95°C for 2 min 30 s and then 35 cycles of 95°C for 20 s, 54°C for 30 s, and 72°C for 45 s, followed by a final extension of 7 min at 72°C. Electrophoresis of the amplified product was performed on a gel containing agarose (1.0% w v^{-1}). Bands on the gel were compared to positive controls of *M. majus* and *M. nivale* that had previously been identified.

Treatments

The trials on *Microdochium* spp., conducted in the growing seasons 2016/17 and 2017/18, included an untreated control and the same 11 seed experimental treatments (see **Table 2** for list of included treatments). The treatments were composed of warm water (WW) at 45°C for 2 h (with and without subsequent drying), WW for 3 h (with and without subsequent drying), four steam treatment regimes (65°C for 90 s, 65°C for 120 s, 70°C for 90 s, 70°C for 120 s), hot air (70°C for 2 days), as well as a biological (Cerall®, Stähler) and a chemical (Celest® Trio, Syngenta) seed treatment. The procedures for the WW, steam, and hot air treatments are described in more detail below. Cerall®, composed of the soil bacterium *P. chlororaphis*, is registered for partial protection against snow mold. The water, steam, and hot air treatment parameters were chosen based on preliminary trials and published studies (Winter et al., 1996; Krebs et al., 2011). In the growing seasons of 2018/19 and 2019/20, only the 2 h WW treatment with subsequent drying was evaluated. The inclusion of only one WW treatment in 2018/19 and 2019/20 was due to consistently good results from the WW treatment in previous years and an interest to focus on steam treatments. A steam treatment of 68°C for 180 s as well as one performed at ThermoSeed Global AB (Uppsala, Sweden) with a ThermoSeed® machine were included for both seasons of 2018/19 and 2019/20, and an additional steam treatment of 70°C and for 180 s was included for the 2019/20 trial. The parameters for the ThermoSeed® machine treatments were determined by ThermoSeed Global AB following preliminary tests and are considered proprietary knowledge. The preliminary testing is carried out to optimize the machine treatment parameters based on the characteristics of the seed lot. All treatments using the same seed were assessed in field trials and laboratory tests with the exclusion of the seed treated for the 2016/17 growing season, which were only tested in the field trial.

The treatments to control loose smut of barley in the growing seasons 2016/17 and 2017/18 included an untreated control and nine experimental seed treatments (see **Table 3** for list of included treatments). The treatments were composed of four WW treatments at 45°C (for 2 or 3 h with or without subsequent drying), three steam treatments (65°C for 90 s, 70°C for 90 s, 70°C for 120 s), hot air, and the synthetic chemical fungicide, Celest® Trio. For the season of 2018/19, only the 2 h WW

treatment with subsequent drying was included as well as two longer steam treatments (68°C for 210 s, 70°C for 210 s). The longer steam treatment times were included to explore the treatment's limit with regard to its phytotoxic effects on barley.

The WW treatments were performed using a flow through water bath, and seeds with a subsequent drying step were laid on a metal tray in a drying oven at 40°C for ∼12 h with circulating air (WTB Binder, Germany). The seed was dried to the original 14% moisture content. The WW treatments without subsequent drying were treated the day prior to sowing and were laid out on an iron mesh overnight at room temperature to allow the seed to partially air-dry. The seed was then sown while still wet (∼25–30% moisture content). The hot air treatment consisted of treating seeds in a drying oven without circulating air (Heraeus, Germany). All non ThermoSeed® steam treatments conducted in seasons 2016/17, 2017/18, and 2018/19 were performed at Sativa Rheinau, using a machine consisting of a conveyor belt and steam humidifier (Condair, Switzerland). The steam treatment for the 2019/20 growing season was performed at Agroscope, Reckenholz, Switzerland using an electric combi-steamer (Model XVC705E-0D00, UNOX). The relative humidity measured in the steam chamber was between 68 and 72%.

Laboratory Tests

In order to directly assess the efficacy of treatments against *Microdochium* spp., seeds included in the field trials in growing seasons 2017/2018, 2018/19, and 2019/20 were examined on agar following a modified ISTA protocol (International Seed Testing Association, 2014). The *Microdochium* infection levels of seed used in the field trials were determined based on 100 seeds on agar plates for the growing season 2017/18 and 200 seeds per treatment for growing seasons 2018/19 and 2019/20. Seeds were surface sterilized for 10 min in a 1% Chloramine T trihydrate solution (Sigma-Aldrich, China), and subsequently, the 100 or 200 seeds were divided into batches of 10 seeds, which were laid out equidistance from each other on a 9 cm diameter Petri plate filled with potato dextrose agar (PDA) (OXOID, United Kingdom) with 0.01% streptomycin (Sigma-Aldrich, China). The seed was then incubated for 7 days at 18°C (±1°C) at 12 h of near ultraviolet light and 12 h of darkness. Outgrowing colonies of *Microdochium* spp. were visually identified based on colony and aerial mycelium appearance using ISTA protocols and as done in other studies focused on detecting *Microdochium* spp. (Kammoun et al., 2009; Matušinsky et al., 2017). Each seed was assessed as either infected or uninfected based on *Microdochium* spp. mycelium growth or its absence around seed. From these data, a percentage of infected seed for each treatment was derived.

Field Trials

Field trials focusing on *Microdochium* spp. in winter wheat were conducted in growing seasons 2016/17, 2017/18, 2018/19, and 2019/20. Field trials focusing on *U. nuda* in winter barley were carried out in growing seasons 2016/17, 2017/18, and 2018/19. All trials were run on experimental fields at Agroscope, Reckenholz (Zurich, Switzerland) and were sown in a randomized complete block design with four replicates (see **Table 1** for additional details). Approximately 1 month prior to sowing, the seedbed was ploughed, and all fields were harrowed with a rotary cultivator within 1 day prior to sowing. Seeds were sown with a Hege 76 disc coulter in 1.5 × 6 m plots with seven rows driven at a speed of ∼1.7 km/h. The plot seeder was equipped with a mechanical rotational distributor that prevented clogging while sowing wet seed, and a set seeding rate was used across all treatments ensuring that the same number of seeds were distributed in the plot. Because the plots had been seeded consecutively, 1.5 m of the plot was destroyed by mulching or glyphosates (0.75 m from each end of the plots' lengths) at the booting stage to avoid any seed treatment mixtures that may have occurred at the ends of the plot. The remaining plot size was 4.5 × 1.5 m. Weather was recorded on a MeteoSwiss weather station located at Zurich-Reckenholz and within 1 km of experimental sites.

Disease Evaluation and Statistical Analysis

The disease assessment for *Microdochium* spp. in field trials was conducted every year in January or February (**Table 1**) by evaluating plant emergence at the growth stage of DC 11 to DC 12 (Zadoks et al., 1974). *Microdochium* spp. primarily affects seedling germination, so the evaluation of field emergence serves as an indicator of treatment efficacy (Vogelgsang et al., 2013). For the field assessment, four counts of plants per meter were averaged within each plot, using areas at least 1 m from the ends of the plot and from inside rows. To test the differences among treatments in each year and trial, ANOVAs were run on the average number of plant per meter within each plot. To see if the data satisfied the assumptions of an ANOVA, quantile comparisons and residuals vs. fitted values were plotted and visually inspected. Additionally, the Levene and Shapiro Wilk tests were run to check assumptions of ANOVAs. The data from the *Microdochium* spp. field trials in years 2016/17, 2018/19, and the trial with the variety Runal in 2019/20 satisfied the assumptions of ANOVA tests. For these years, a Tukey's honest significant difference (HSD) test was used for pairwise comparisons following significant ANOVA results. The data from the *Microdochium* spp. trials in 2017/18 and 2019/20 on the variety Nara exhibited a non-parametric distribution, and so a Kruskal Wallis test was used followed by the Pairwise Wilcoxon Rank Sum test with the Benjamini & Hochberg procedure to adjust for multiple comparisons.

Analyses of seeds set on agar plates in the laboratory were also used to determine *Microdochium* spp. infection in three of the growing seasons 2017/18, 2018/19, and 2019/20. To examine percentage of diseased seeds on agar plates, the difference in numbers of seeds with and without *Microdochium* spp. colonies were evaluated using the Fisher's Exact Test. Additionally, *post-hoc* pairwise tests of independence for nominal data was conducted to look for pairwise differences between treatments.

For loose smut of barley, the infection rate was assessed by counting the number of infected heads within a set area of the plot that differed between years to account for variable infection rates. Because of a very low infection rate in 2017, all infected heads per plot were counted. Due to a much higher infection rate in years 2018 and 2019 (**Figure 2**), infected heads

were counted along a random 1 m transect. In all years, the *U. nuda* infection was evaluated before flowering (DC 59). In all growing seasons, the number of plants along the 1 m transect was also counted to help determine if any treatments had a phytotoxic effect and inhibited plant growth. The loose smut data from the growing season 2018/19 satisfied the assumptions of ANOVAs and were evaluated using Tukey's HSD following a significant ANOVA result. For the two other years, the assumptions of homogeneous variances and normal distribution were violated, so the non-parametric Kruskal-Wallis test was performed followed by a Pairwise Wilcoxon Rank Sum test with the Benjamini & Hochberg procedure to adjust for multiple comparisons. All statistical analyses and graphs were carried out in R (version 4.0.3) using the following R packages: car (Fox and Weisberg, 2019), multcompView (Graves et al., 2015), ggplot2 (Wickham, 2016), and rcompanion (Mangiafico, 2016).

Weather Conditions

Weather conditions in the 2016/17 season were extremely dry and devoid of snow. Precipitation totals from December 2016 to February 2017 were half of the normal values based on average totals from 1981 to 2010 (MeteoSchweiz, 2017, 2018, **Supplementary Figure 1**). The spring was also exceptionally warm. In the 2017/18 season, record warm temperatures were measured in January, but February was very cold, followed by a cool March and warm spring (MeteoSchweiz, 2019). Precipitation in January was very high compared to the normal levels followed by drier than normal spring. In December of the 2018/2019 growing season, it was particularly wet with over 150 mm of rain—nearly double the norm. February to April showed slightly warmer and drier than average temperatures and precipitation (MeteoSchweiz, 2020). The 2019/2020 growing season had a more mild winter with slightly more precipitation than average over the winter months and slightly warmer temperatures (MeteoSchweiz, 2021).

RESULTS AND DISCUSSION

Microdochium spp.

The seed used in the field and laboratory trials was infected with a mixture of *M. nivale* and *M. majus* based on the presence of bands after the amplification of DNA using a species-specific primer set for each pathogen. These two species often co-exist (Nielsen et al., 2013), but previous work suggests that *M. majus* has a selective advantage over *M. nivale* on wheat, where it is typically more prevalent than *M. nivale* (Simpson et al., 2000). These findings correspond to the stronger bands we found in all of our seed samples while using the primer set for *M. majus*. Nonetheless, it is thought that the differentiation of *M. majus* and *M. nivale* may not be essential from a disease control and economic standpoint due to their widespread co-occurrence (Nielsen et al., 2013).

Because a seed-borne infection with *Microdochium* spp. primarily affects the germination ability of the seed, seedling emergence, and plant establishment (Nielsen et al., 2013; Vogelgsang et al., 2013), emergence rate was used to measure infection levels in field trials. Across all four years, the seed treatment had a significant effect on plant emergence in the field trial [2016/17: $F_{(11,36)} = 6.51$, $p < 0.01$; 2017/18: $H_{(11)} = 43.23$, $p < 0.01$; 2018/2019: $F_{(11,36)} = 46.16$, $p < 0.01$; 2019/20-Nara: $H_{(4)} = 17.72$, $p = 0.01$; 2019/2020-Runal: $F_{(4,15)} = 12.76$, p-value < 0.01], showing that the treatments had different effects on the control of *Microdochium* spp. In all trials, WW treatments showed the greatest plant emergence (**Figure 1**), and all WW treatments resulted in significantly more plant emergence than in the control treatments. Previous experiments have shown the WW results to be on par with those of synthetic chemically treated seed (Winter et al., 1994) as observed in this study (**Table 2**). In 2016/17 and 2017/18, the four variations of the WW treatment (2 h with and without drying, 3 h with and without drying) yielded similar levels of plant emergence with the exception of a decrease in the 2017/18 2 h WW without drying treatment (**Figure 1**). However, the 2 h WW

TABLE 2 | The detection of *Microdochium* spp. in winter wheat seeds following treatments included in the field trials between 2017/18 and 2019/20.

Growing season	Treatment	Variety and harvest year	Type of treatment	Percentage of seeds infected with *Microdochium* spp. (%)[1]
2017/18	Untreated control	Nara 2017	Control	52^{d}
	WW, 2 h, dried		Warm water	2^{ab}
	WW, 2 h, wet		Warm water	7^{b}
	WW, 3 h, dried		Warm water	0^{a}
	WW, 3 h, wet		Warm water	0^{a}
	Steam 65°C, 90 s		Steam	44^{d}
	Steam 65°C, 120 s		Steam	42^{d}
	Steam 70°C, 90 s		Steam	26^{c}
	Steam 70°C, 120 s		Steam	19^{c}
	Hot air, 2 d		Hot air	26^{c}
	Cerall®		Biological	47^{d}
	Celest® Trio		Chemical	5^{ab}
2018/19	Untreated control	Nara 2017	Control	37^{b}
	WW, 2 h, dried		Warm water	8^{a}
	ThermoSeed®		Steam	16^{a}
	Steam 68°C, 180 s		Steam	7^{a}
	Celest® Trio		Chemical	7^{a}
2019/20	Untreated control	Nara 2017	Control	46^{d}
	WW, 2 h, dried		Warm water	4^{a}
	ThermoSeed®		Steam	29^{c}
	Steam 68°C, 180 s		Steam	12^{b}
	Steam 70°C, 180 s		Steam	20^{c}
2019/20	Untreated control	Runal 2019	Control	25^{c}
	WW, 2 h, dried		Warm water	2^{a}
	ThermoSeed®		Steam	10^{b}
	Steam 68°C, 180 s		Steam	12^{b}
	Steam 70°C, 180 s		Steam	12^{b}

Results show the percentage of seed infected with Microdochium spp. using the agar plate detection method (N = 100 seeds for 2017/18 and 2018/19 and N = 200 seeds for 2019/20).

[1] *The letters associated with percentage of seed infected with Microdochium spp. indicate significant differences based on Fisher's Exact Test ($\alpha = 0.05$). Tests were adjusted for multiple comparisons using the Benjamini & Hochberg method.*

TABLE 3 | Field trial results focusing on loose smut of barley (*Ustilago nuda*) control, showing the number of barley plants per meter from 2016/17 to 2018/19.

Growing season	Treatment	Variety and harvest year	Type of treatment	Number of plants per meter (mean ± sd) (%)[1]
2016/17	Untreated control	Cassiopee 2016	Control	58 ± 2^{ab}
	WW, 2 h, dried		Warm water	58 ± 10^{ab}
	WW, 2 h, wet		Warm water	64 ± 5^{a}
	WW, 3 h, dried		Warm water	51 ± 5^{b}
	WW, 3 h, wet		Warm water	67 ± 4^{a}
	Steam 65°C, 90 s		Steam	56 ± 3^{ab}
	Steam 70°C, 90 s		Steam	59 ± 8^{ab}
	Steam 70°C, 120 s		Steam	63 ± 4^{ab}
	Hot air, 70°C, 2 d		Hot air	67 ± 3^{a}
	Celest® trio		Chemical	62 ± 4^{ab}
2017/18	Untreated control	Caravan 2015	Control	53 ± 4^{ab}
	WW, 2 h, dried		Warm water	43 ± 3^{b}
	WW, 2 h, wet		Warm water	48 ± 3^{ab}
	WW, 3 h, dried		Warm water	52 ± 4^{ab}
	WW, 3 h, wet		Warm water	52 ± 3^{ab}
	Steam 65°C, 90 s		Steam	58 ± 8^{a}
	Steam 70°C, 90 s		Steam	55 ± 4^{a}
	Steam 70°C, 120 s		Steam	49 ± 2^{ab}
	Hot air, 70°C, 2 d		Hot air	53 ± 3^{ab}
	Celest® trio		Chemical	52 ± 9^{ab}
2018/19	Untreated control	Caravan 2018	Control	73 ± 8^{a}
	WW, 2 h, dried		Warm water	65 ± 48^{a}
	Steam 68°C, 210 s		Steam	70 ± 3^{a}
	Steam 70°C, 210 s		Steam	50 ± 3^{b}
	Untreated control		Steam	73 ± 4^{a}

The infection rate in the field from these treatments are shown in ***Figure 2****.*

[1] *Different letters indicate significant Tukey's HSD differences between seed treatments ($\alpha = 0.05$) for all comparisons. Tests were adjusted for multiple comparisons using the Benjamini & Hochberg method.*

without drying treatment in 2016/17 gave a slightly higher plant emergence rate, although not significantly different from the other WW treatments. Across both years, the majority of the WW treatments with and without subsequent drying showed no difference in treatment outcome, suggesting that the sowing of wet seed soon after the treatment did not negatively affect plant germination and growth. Wetting seed prior to planting can give them a priming effect (Harris, 2006), especially in salinity-stressed or dry climates (Bouaziz and Bruckler, 1989; Carrillo-Reche et al., 2018). Because the experiments were conducted in a humid continental climate and because measurements were taken at DC 11 to DC 12, giving plant sufficient time to germinate, it was assumed that the main observed differences in plants per meter among treatments were due to disease reduction. Nonetheless, there are reports of synergies between priming effects and disease reduction (Musa et al., 2001; Harris, 2006), so a positive effect of priming due to seed imbibition may also contribute to the observed treatment differences.

The corresponding decrease of *Microdochium* spp. infections in treated seed based on the laboratory results reflects the differences in wheat emergence rate in the field for most treatments. Some phytotoxic effects of the higher temperature steam treatments are evident based on a lower germination rate in the field despite a decreased *Microdochium* spp. infection rate observed in the laboratory (i.e., the steam treatment at 68°C for 180 s in 2018/19 trial—discussed in more detail below). WW treatments have previously shown good effectiveness against *Microdochium* spp., and various time and temperature parameters have been explored (Winter et al., 1997, 1998a). Based on these previous results, 45°C was found to be most effective while minimizing phytotoxic effects, which were observed more often while using hot water (52°C) with shorter treatment times (Winter et al., 1998b). The phytotoxic effects become more noticeable at the 52°C WW treatment because seeds are more sensitive to higher temperatures at elevated moisture contents (Bewley et al., 2013; Tangney et al., 2019). This relationship between temperature tolerance and seed moisture content corresponds to the use of higher temperatures in steam and hot air treatments. The moisture content of seeds treated with steam or hot air is less than that of seeds treated in a water bath, and therefore, they can withstand higher temperatures.

In 2016/2017 and 2017/18, the hot air treatment showed a significant improvement over the untreated control, but it was not as effective as the WW treatment (**Figure 1**). Based on the results from the agar plate detection tests (**Table 2**), both hot air and WW treatments showed a reduction in *Microdochium* spp. The hot air, consisting of dry heat, has also shown good results on other seed-borne diseases, including bacterial blight caused by *Pseudomonas syringae* pv. *Pisi* in pea seeds (Grondeau et al., 1992) as well as a number of viral diseases (Koch and Roberts, 2014). On the other hand, Cerall® showed no improvement in plant emergence over the untreated control treatment in our 2016/17 and 207/28 field trials. The agar plate detection results of the Cerall® treated seeds from the 2017/18 season showed no significant reduction of *Microdochium* spp., indicating that the product in this study was not effective against the disease. This finding is in line with results from Johnsson and colleagues, who found limited effect of the active bacterium in Cerall®, *P. chlororaphis* MA 342, against *Microdochium* spp. in spring and winter wheat.

Overall, steam has the potential to be very effective at reducing *Microdochium* spp. infection, but its performance in the field within this study was dependent on the year—most likely due to a combination of seed quality, as well as field and weather conditions. In 2016/17, there were no differences between any of the steam treatments and the untreated control (**Figure 1**). In 2017/18, the 65°C 120 s treatment showed improvement over the control while the other treatments did not. In 2018/19, both the ThermoSeed® treatment and other steam treatments had poor results; they either showed no difference from the untreated control or reduced plant emergence, respectively. This was most likely a combination of challenging field conditions and low seed quality. Prior to treatment, ThermoSeed Global AB had advised against using the Nara 2017 seed lot because their preliminary tests suggested a possibility of poor seed condition and, thus, poor performance. Nevertheless, the Nara lot was used to assess the differences of treatments in a more challenging

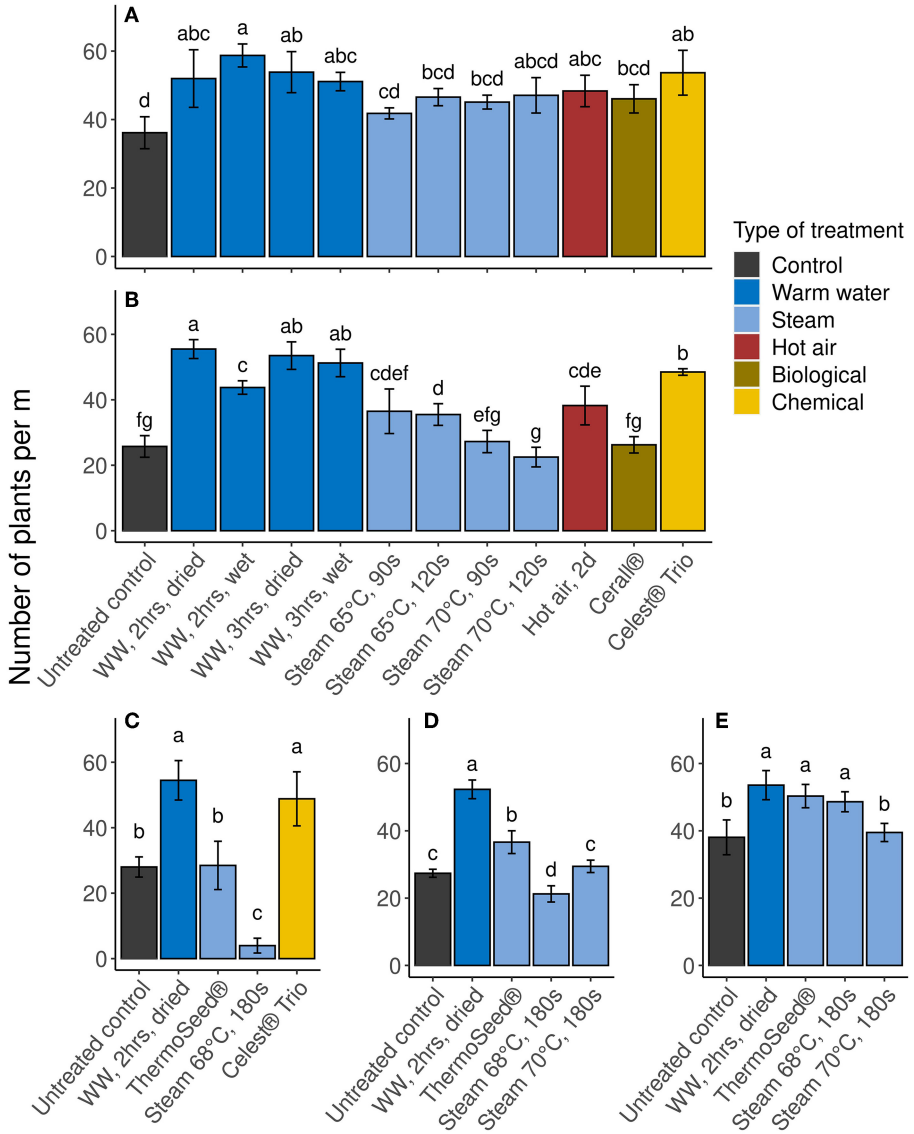

FIGURE 1 | Efficacy of thermal and alternative treatments to control *Microdochium* spp. in wheat under field conditions based on field germination rates (number of plants per meter) in **(A)** 2016/17, **(B)** 2017/18, **(C)** 2018/19, **(D)** 2019/20 with the variety Nara, and **(E)** 2019/20 with the variety Runal. Treatments included warm water (WW), steam, hot air, and biological (Cerall®) and chemical (Celest® Trio) plant protection products. Times in thermal treatments indicate different treatment durations. Error bars indicate the standard deviation of the means. Means sharing a letter are not significantly different ($\alpha = 0.05$).

seed lot and to test the limitations of the treatment. Indeed, the steam treatments on the Nara seed lot did not perform well in 2018/19. This was most likely due to the interaction of the high infection rate and poor physiological condition of the seed as well as the poor field conditions. Due to especially high amount of rainfall and very wet conditions after sowing (**Supplementary Figure 1**), the field became flooded in 2018/19, making it especially challenging for the more heavily infected Nara seed lot. Waterlogging can particularly affect plants at the germination and emergence phase (Setter and Waters, 2003), and these challenging conditions combined with highly infected seed appeared to cause increased mortality to the plants at these stages in our study. Previous work has also noted the interaction of treatment effect and weather conditions (Osman et al., 2004), suggesting that the poor weather conditions were too difficult for the highly infected seed to overcome. Due to the additional stress from the thermal treatment in combination with the field and seed condition, the 68°C, 180 s steam treatment showed reduced germination compared to the control.

To examine the performance of the same Nara seed lot under better field conditions, the ThermoSeed® and the 68°C, 180 s steam treatments were repeated in 2019/20 with an additional stream treatment at 70°C. Indeed, the ThermoSeed® treatment in 2019/20 showed higher plant emergence than the untreated control treatment, while the 68 and 70°C steam treatments performed the same or worse compared to the

untreated control. For the Runal seed lot, both the ThermoSeed® and 68°C steam treatment in 2019/20 showed significantly higher plant emergence than the untreated control, while the 70°C steam treatment showed no improvement. The agar plate detection results demonstrated disease reduction in all steam treatments over 65°C and a marginal decrease in the 65°C treatments (**Table 2**). Therefore, it appears that the reduced plant emergence observed in some of the steam treatments was due to phytotoxic damage to the seed rather than from infection with *Microdochium* spp. Phytotoxic effects resulting in reduced germination from steam treatments and dry heat treatments have been documented (Koch and Roberts, 2014), illustrating the usefulness of establishing optimal steam treatment parameters for each lot. Due to the potential for phytotoxic effects, the implementation of steam treatments in practice perform better following preliminary tests (Forsberg et al., 2003; Forsberg, 2004). Like the other thermal treatments, water has the potential to reduce germination, as previously found when the higher temperature of 52°C is used (Winter et al., 1996). Based on this work, the 2 h, 45°C WW treatment was found to result in minimal phytotoxic effects.

Ustilago nuda

In the *U. nuda* trial on barley, significant differences were found in all years among treatments [2016/17: $H_{(9)} = 34.77$, $p < 0.01$; 2017/18: $H_{(9)} = 34.19$, $p < 0.01$; 2018/2019: $F_{(4,15)} = 16.29$. $p < 0.01$]. Only the WW and chemical control treatments showed a consistent and significant reduction of diseased heads in the field (**Figure 2**). WW treatments have previously shown effectiveness against *U. nuda* (Batts, 1956; Doling, 1965). Interestingly, the chemical control showed a decreased effectiveness compared to the WW in the growing seasons 2017/18 and 2018/19. Resistance of *U. nuda* to other chemical fungicides that include carboxamides has been documented (Leroux and Berthier, 1988; Dhitaphichit and Jones, 1991; Menzies et al., 2005). However, *U. nuda* fungicide resistance was not assessed in this experiment. The WW treatment did not show any significant phytotoxic damage to the seed compared to the untreated control based on the number of plants per meter (**Table 3**). The steam 65°C, 90 s treatment in 2016/17 showed a significant improvement over the untreated control without a significant reduction in number of plants, but in this year, the rate of disease was relatively low. In 2018/19, the strongest steam treatment (70°C, 210 s) was not effective enough to significantly reduce the infection rate but, nonetheless, was accompanied by a severe reduction in the number of plants, suggesting a phytotoxic effect. Based on the results presented in the current study and previous studies (Doling, 1965; Winter et al., 1996), WW was the only seed treatment to work effectively and consistently against *U. nuda*.

PRACTICAL CONSIDERATIONS AND IMPLICATIONS

The results from our field and laboratory trials to control *Microdochium* spp. and field trials to control *U. nuda* suggest that different alternative treatments are better suited for the diseases included in this study. The steam treatment can be very effective against *Microdochium* spp. as well as other seed-borne diseases, including *T. caries* (Forsberg et al., 2005). In the present study, steam was generally not effective against *U. nuda* with the exception of one steam treatment regime (steam 65°C, 90 s) in the 2016/17 season. The difficulty in using steam to combat *U. nuda* may be due to the pathogen's heat resistance or localization in the embryo, which makes it difficult to kill the pathogen without damaging the seed. Further work is needed to disentangle these two factors. Cerall® was found to be ineffective against *Microdochium* spp., which corroborates previous work (Johnsson et al., 1998). However, Cerall® was shown to be more effective against *T. caries* (Hökeberg et al., 1997), agreeing with its primary intended use according to its registration.

In this study, WW treatments were found to be effective against two diseases with diverse localities within seeds. However, previous work has shown only moderate effects of WW treatments against *T. caries* (Winter et al., 1998b), so it would not be optimal for all seed-borne diseases. Nonetheless, *U. nuda* is especially challenging to treat with alternative treatments due to its presence within the seed embryo. Previous research exploring various seed treatments with plant extracts and the bacterial biocontrol agent, *P. chlororaphis*, found insufficient success against *U. nuda* (Tombolini et al., 1999; Koch et al., 2010). The WW treatments used in the present study showed effective reduction of *U. nuda* and *Microdochium* spp. without adverse effects on the seed quality.

The introduction of WW as a prophylactic seed treatment for cereals would be extremely challenging due to the logistical and technical constraints associated with implementing it on a large-scale and the comparatively low value of cereal seeds. The energy requirement associated with re-drying the seed is one main obstacle (Borgen, 2004; Matanguihan et al., 2011), and many economic questions remain regarding its adoption (Osman et al., 2004). If an on-farm WW treatment option were available, the direct sowing of wet seed could offer a way to overcome the energy requirement for re-drying WW-treated seeds. Our results suggest that a WW treatment without subsequent drying could be a valid treatment option on a small-scale. This type of treatment may be appropriate for small organic producers or in special cases when there is a desire to propagate known *U. nuda* infected seed, but more work is needed to evaluate which equipment is suitable to sow wet seed. We have sown wet, but air-dried overnight, WW treated seed in an one hectare field trial unrelated to the present study with an Amazone D8-30 Special drill seeder (unpublished data). This type of seed driller is used in normal farm operations, whereas the plot seeder used in this study is usually only used for research purposes. For the one hectare trial, the sowing rate was reduced due to a decreased flow, however, clogging was not a problem. Therefore, the sowing of wet seed may require adaptation to farm equipment and could be tested with different types of seeders. For example, pneumatic seeders may be more capable to handle wet seed. The use of on-farm seed priming is appropriate for resource-poor, small-scale farmers (Harris, 2006), suggesting the feasibility, on a small-scale, of sowing wet, surface-dried seed. The incorporation of warm water

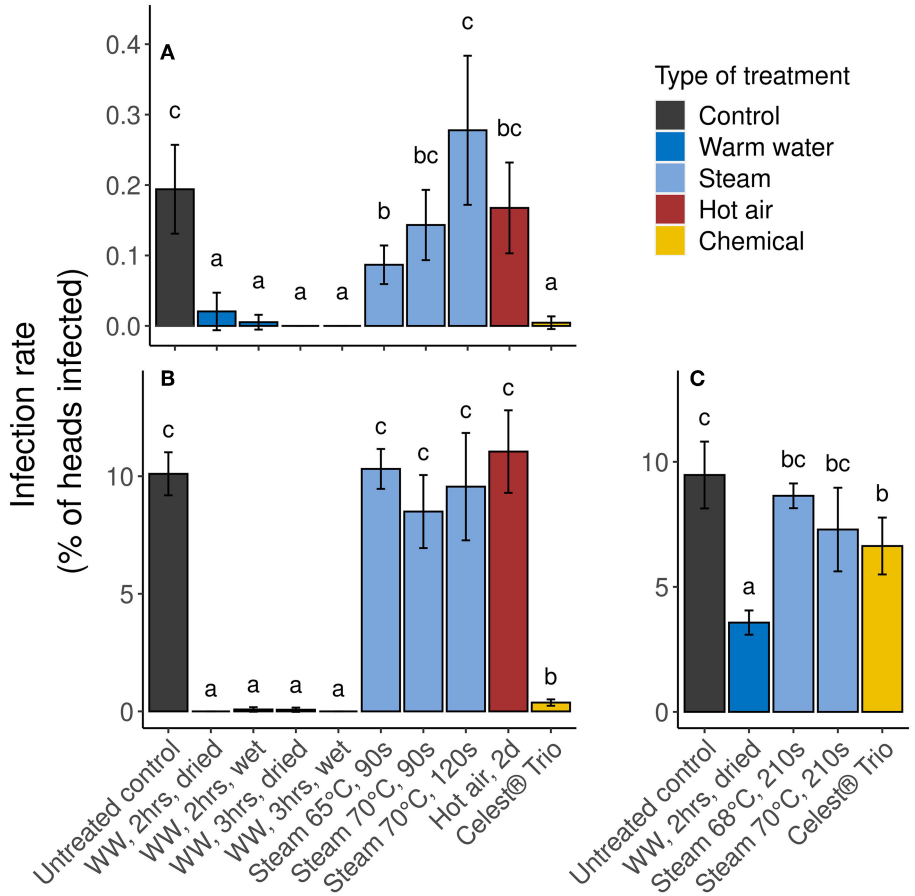

FIGURE 2 | Efficacy of thermal treatments to control *Ustilago nuda* in barley based on percentage of infected heads from field trials in **(A)** 2016/17, **(B)** 2017/18, and **(C)** 2018/19. Different y-axis scales are used in **(A)** than those used in **(B,C)** to accomodate different infection rates. Times in thermal treatments indicate different treatment durations. Error bars show the standard deviation of the means. Means sharing a letter are not significantly different ($\alpha = 0.05$).

in such treatments on a small-scale may be suitable in particular cases to combat *U. nuda* due to the lack of other effective alternative treatments against loose smut or for early generations of barley multiplication (pre-basic and basic seed). Further work and economic analysis are needed to determine when it would be advisable.

Steam, on the other hand, which is effective against many seed-borne diseases, but not *U. nuda*, is easier to implement on a larger scale. Steam has a reduced energy and water requirement compared to the WW treatment, lowering treatment costs (Sharma et al., 2015). The reduced water and energy consumption required for steam compared to hot water also makes steam advantageous for thermal weed control (Ascard et al., 2007). The use of steam for seed treatment is also more feasible because driers can be integrated into the treatment, enabling a flow-through process (Forsberg et al., 2005). The seed has a lower moisture content following steam treatment, easing the drying procedure compared to a warm water treatment. The use of preliminary tests for each seed lot is necessary to minimize possible detrimental effects to the seed. Seed lots with low thermal tolerances due to seed age, handling or storage conditions can be difficult to treat with steam, and such pre-tests can determine the steam treatment's suitability (Forsberg et al., 2003). Due to the reduction of germination that can result from the thermal treatments' damage to the seed, the goal is to find a balance between minimizing injury to the plant and maximizing disease reduction.

CONCLUSIONS

Our field trials suggest that, in general, thermal seed treatments show promise for Swiss agriculture. The implementation of these treatments is dependent on a number of factors, including ease of adoption, characteristics of the diseases to be controlled, and energy requirements. Additional work in diverse field and ecological conditions across Switzerland will further establish the effectiveness of alternative seed treatments and the ability to implement them into the Swiss market. Due to different efficacies of diverse alternative treatments across diseases, improved fungal pathogen detection methods will assist decision making regarding

disease control (Vannacci et al., 2014). Such a testing strategy could match effective treatments with seed lots depending on disease presence and infection levels and, ultimately, reduce prophylactic seed dressings with synthetic chemical fungicides.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

AUTHOR CONTRIBUTIONS

IB and AK: conceptualization. KES and SK: data analysis. IB, AK, and SK: investigation. KES: writing—original draft preparation and visualization. SV, AB-M, and KES: writing—review and editing. KES and AB-M: project administration. SV, TH, KES, and AB-M: funding acquisition. All authors have read and agreed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2021.775243/full#supplementary-material

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