Phytoprotection

Efficacy of acetic acid vapours and dry heat to control *Fusarium graminearum* and *Bipolaris sorokiniana* in barley and wheat seeds

Efficacité des vapeurs d'acide acétique et de la chaleur sèche pour réprimer *Fusarium graminearum* et *Bipolaris sorokiniana* dans les semences d'orge et de blé

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Résumé de l'article

Dans le but de trouver un traitement de semences acceptable en production céréalière biologique, nous avons testé l'efficacité de trois traitements non chimiques, soit la chaleur sèche ainsi que les vapeurs d'acide acétique à faible dose (VAA-L) et à forte dose (VAA-H), pour contrer le *Fusarium graminearum* (*Fg*) et le *Bipolaris sorokiniana* (*Bs*), deux agents pathogènes qui affectent la levée et le rendement de l'orge et du blé lorsque présents sur les semences. Ces traitements ont été comparés à un témoin sans traitement et au Vitaflo®-280. Les traitements ont été appliqués sur six lots de semences d'orge et six lots de blé contaminés par *Fg* à plus de 20 % (c'est-à-dire, > 20 % de grains contaminés) ou par *Bs* à plus de 50 %. Pour tous les lots contaminés par *Fg*, les trois traitements non chimiques ont réduit la contamination sous le seuil de nuisibilité de 15 %, soit le seuil recommandé au Danemark pour *Fusarium* spp. Dans les lots contaminés par *Bs*, VAA-H a réduit la contamination dans le plus grand nombre de lots, suivi par VAA-L, puis par la chaleur sèche, laquelle n'a eu aucun effet chez l'orge. Cependant, ces traitements n'ont pas réduit la contamination par *Bs* sous le seuil de nuisibilité de 30 %, à l'exception de VAA-H dans un lot d'orge et de la chaleur sèche dans un lot de blé. VAA-H a aussi réduit la germination dans trois lots de blé et dans le lot d'orge nue AC Hawkeye, ce qui a eu des répercussions négatives sur le rendement en grains dans deux de ces lots de blé. VAA-H n'a pas eu d'effet sur le rendement dans les autres lots et les autres traitements n'ont eu d'effet sur aucun lot. La chaleur sèche s'est montrée efficace pour réduire *Fg* sur les deux céréales, alors que VAA-H a démontré un certain potentiel pour réduire *Fg* et *Bs*, mais chez les espèces à grains vêtus seulement. Aucun des traitements évalués ne semble convenir pour réduire les deux agents pathogènes à la fois sur le blé et sur l'orge.

Efficacy of acetic acid vapours and dry heat to control *Fusarium graminearum* and *Bipolaris sorokiniana* in barley and wheat seeds

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To find seed treatments that are acceptable for organic cereal production, we tested the efficacy of three treatments, i.e. dry heat, a low dose of acetic acid vapours (AAV-L), and a high dose of AAV (AAV-H), to control *Fusarium graminearum* (*Fg*) and *Bipolaris sorokiniana* (*Bs*), two seed-borne pathogens affecting emergence and yield in barley and wheat. These treatments were compared with a control (no treatment) and Vitaflo®-280. Treatments were applied on six barley and six wheat seed lots contaminated with *Fg* at a rate of > 20% (i.e. > 20% of seeds contaminated) and/or *Bs* at a rate of > 50%. For all *Fg*-contaminated lots, the three non-chemical treatments reduced the contamination rate under the rejection threshold of 15%, which is the Danish recommendation for *Fusarium* spp. For *Bs*-contaminated lots, AAV-H reduced contamination the most, followed by AAV-L, and then by dry heat, which had no effect on barley. However, these treatments did not reduce *Bs* contamination under the rejection threshold of 30%, except for AAV-H in one barley lot and dry heat in one wheat lot. Also, AAV-H reduced the germination in three wheat lots and in the hulless barley AC Hawkeye, and this had negative effects on grain yield for two of the wheat lots. AAV-H had no effect on grain yield in the other lots, and neither did the other treatments in any of the lots. Dry heat was effective for controlling *Fg* in both cereals, whereas AAV-H showed some potential to control both pathogens, but only in covered grains. None of the treatments evaluated appears to be appropriate for reducing contamination by either pathogens in wheat and barley.

Keywords: acetic acid vapour, barley, *Bipolaris sorokiniana*, dry heat, *Fusarium graminearum*, *Hordeum vulgare*, seed treatments, *Triticum aestivum*, wheat

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Dans le but de trouver un traitement de semences acceptable en production céréalière biologique, nous avons testé l'efficacité de trois traitements non chimiques, soit la chaleur sèche ainsi que les vapeurs d'acide acétique à faible dose (VAA-L) et à forte dose (VAA-H), pour contrer le *Fusarium graminearum* (*Fg*) et le *Bipolaris sorokiniana* (*Bs*), deux agents pathogènes qui affectent la levée et le rendement de l'orge et du blé lorsque présents sur les semences. Ces traitements ont été comparés à un témoin sans traitement et au Vitaflo®-280. Les traitements ont été appliqués sur six lots de semences d'orge et six lots de blé contaminés par *Fg* à plus de 20 % (c'est-à-dire, > 20 % de grains contaminés) ou par *Bs* à plus de 50 %. Pour tous les lots contaminés par *Fg*, les trois traitements non chimiques ont réduit la contamination sous le seuil de nuisibilité de 15 %, soit le seuil recommandé au Danemark pour *Fusarium* spp. Dans les lots contaminés par *Bs*, VAA-H a réduit la contamination dans le plus grand nombre de lots, suivi par VAA-L, puis par la chaleur sèche, laquelle n'a eu aucun effet chez l'orge. Cependant, ces traitements n'ont pas réduit la contamination par *Bs* sous le seuil de nuisibilité de 30 %, à l'exception de VAA-H dans un lot d'orge et de la chaleur sèche dans un lot de blé. VAA-H a aussi réduit la germination dans trois lots de blé et dans le lot d'orge nue AC Hawkeye, ce qui a eu des répercussions négatives sur le rendement en

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grains dans deux de ces lots de blé. VAA-H n'a pas eu d'effet sur le rendement dans les autres lots et les autres traitements n'ont eu d'effet sur aucun lot. La chaleur sèche s'est montrée efficace pour réduire *Fg* sur les deux céréales, alors que VAA-H a démontré un certain potentiel pour réduire *Fg* et *Bs*, mais chez les espèces à grains vêtus seulement. Aucun des traitements évalués ne semble convenir pour réduire les deux agents pathogènes à la fois sur le blé et sur l'orge.

Mots-clés : *Bipolaris sorokiniana*, blé, chaleur sèche, *Fusarium graminearum*, *Hordeum vulgare*, orge, traitements de semences, *Triticum aestivum*, vapeur d'acide acétique

INTRODUCTION

Fusarium graminearum Schawbe [*Gibberella zeae* (Schwein.) Petch] and *Bipolaris sorokiniana* [*Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur] are two major pathogens of cereal seeds that cause damping off and root rot in Canada (Christensen and Stakman 1935; Pouleur *et al.* 2006). Yield losses associated with those diseases can reach 6-7% for wheat (*Triticum aestivum* L.) and 10% for barley (*Hordeum vulgare* L.) (Bailey *et al.* 2003), and would be attributable to a reduction in seedling emergence and to the lower yield of surviving plants that are weakened by the diseases (De Tempe 1964). Poor seedling emergence and stand establishment result in poor weed competition and uneven ripening (O'Donovan *et al.* 2007, 2008). In organic farming, fast and even emergence is essential for the crop to compete with weeds. In Denmark, organic farmers are advised not to use seed lots contaminated more than 15% with *Fusarium* spp. and 30% by *B. sorokiniana* (Nielsen 2000). However, some years, it may be difficult to find seed lots below those contamination thresholds, especially under the humid conditions of eastern Canada. In such cases, seed treatment with fungicides can help to reduce the impact of seedling diseases in conventional farming systems, but in organic farming, chemical products are not allowed and biocontrol agents are not available as cereal seed treatment in Canada. Increasing the planting rate can improve barley stands' competitiveness (O'Donovan *et al.* 2008), and this may be a good strategy for seed lots that are above the recommended contamination thresholds. However, planting a greater proportion of infected seeds may not be helpful as it results in the presence of more pathogen inoculum in the field (Saskatchewan Ministry of Agriculture 2012). In the organic farming context, there is a need to look for alternative seed treatments to improve seed phytosanitary quality and to limit the impact of seed-borne diseases on seed germination, seedling growth and stand establishment.

Before the arrival of synthetic molecules, many treatments were tested for their capacity to reduce the presence of pathogens in cereal seeds. The first studies on seed treatments in cereals were published in the 19th century (Forsberg *et al.* 2005). Thermotherapy, which includes hot water, dry heat, moist heat and radiation, has been used to control seed-borne pathogens (Agarwal and Sinclair 1987). Other methods have included the use of plant extracts or other liquids, such as acetic acid (Nielsen *et al.* 2000).

More recently, with the development of organic agriculture resulting from social pressure to reduce pesticide use, studies investigating alternatives to fungicides have been reported. Thermotherapy techniques and physical methods have been studied or are currently in use in some countries (Borgen 2004a, 2004b; Gaurilčikiené *et al.* 2013). Such techniques include water vapour, hot moist air, ozone, electro-rays, microwave and ultrasound. Seed coating with various natural products including plant extracts or biological control agents such as Cedomon (*Pseudomonas chlororaphis*) are also accepted for organic farming in some European countries (Borgen 2004a).

Volatile organic compounds have been reported to inhibit *in vitro* mycelial growth of *Sclerotinia sclerotiorum* (Lib.) de Bary in bean (*Phaseolus vulgaris* L.) seeds artificially infested with this pathogen (Fialho *et al.* 2011). Other recent studies have focused on semiochemicals that favour plant defense mechanisms. Those compounds have been shown to induce long-lasting resistance to *Botrytis cinerea* Pers.:Fr. in tomato plants (*Solanum lycopersicum* L.) grown from seeds treated with those semiochemicals (Worrall *et al.* 2012). As the defense response induced is not accompanied by plant growth reductions, semiochemicals may be considered for seed treatment in cereals to reduce fusarium crown and root rot (Wenda-Piesik *et al.* 2010).

Many of the techniques used in cereals can reduce seed contamination by *Fusarium* spp. However, to our knowledge, no studies have focused on *B. sorokiniana* because it is less of a widespread problem (Borgen 2004a). In Canada, the application of biocontrol agents such as *Clonostachys rosea* to reduce seed-borne pathogens on wheat seeds has also been studied (Xue *et al.* 2007). Other work conducted on wheat and barley seed lots has shown that dry heat treatments are effective to control seed-borne populations of *F. graminearum*, but rather ineffective against *B. sorokiniana* (Couture and Sutton 1980; Clear *et al.* 2002). Treatments with acetic acid vapours (Sholberg *et al.* 2006) have been developed to treat cereal seeds. Preliminary tests (Pouleur *et al.* 2008) showed that acetic acid vapours were able to reduce viable *F. graminearum* populations in wheat and barley seeds without affecting seed germination. Although this treatment had less effect on *B. sorokiniana*, a higher dose could be more effective.

The objective of the present study was to evaluate, in wheat and barley, the efficacy of two doses of acetic acid vapours and dry heat as seed treatments to reduce the impact of the seed-borne pathogens *F. graminearum* and *B. sorokiniana*.

MATERIALS AND METHODS

From 2010 to 2012, five seed treatments were tested: a low dose of acetic acid vapours (AAV-L); a high dose of acetic acid vapours (AAV-H); dry heat; Vitaflo®-280 (carbathiin/thiram) as a standard chemical control; and an untreated control. Each year, seed treatments were applied on two barley and two wheat seed lots contaminated with *F. graminearum* at a contamination rate of > 20% and/or *B. sorokiniana* at a rate of > 50%. Six contaminated barley lots and six contaminated wheat lots were evaluated over 3 yr (2010-2012). The lots were harvested in the province of Quebec from 2007 to 2011. The seven spring cultivars chosen for the study are well adapted to growing conditions in eastern Canada, and most of them are used in organic farming. The characteristics of the lots used in the present study are presented in Table 1. Their germination rates varied from 66.5% to 90.8% (Tables 2 and 3; control treatment). Treatments were applied on subsamples of 200 or 300 g. There were four subsamples per treatment per lot, and the four subsamples constituted the four blocks of the randomized complete block design.

Treatments

For acetic acid vapour treatments, three 300 g subsamples from the same seed lot and from the same block were placed directly in three 23 L chambers (one subsample per chamber): one for the low dose of acetic acid, one for the high dose, and one with no acetic acid that was used as control in which a humidity/temperature probe was placed. The latter sample was not included in the study since it was only used to monitor relative humidity (RH) and temperature in the two other chambers in which no probe could be added as acetic acid affects it. Sterile distilled water was added to all the chambers, and RH and temperature were monitored in the control chamber. The assumption was that all chambers were similar and therefore so were RH and temperature. When RH remained constant (i.e. at approximately 50 to 60%), acetic acid was added to the treatment chamber. An initial amount of acetic acid was added at the start of the treatment and additional amounts were added as required to maintain the amount of acetic acid vapour above approximately 10 mg L^{-1} of headspace for the low dose and 20 mg L^{-1} for the high dose. The level of acetic acid in the chamber was monitored using a gas chromatograph. After 120 min of treatment, the chambers were vented, and the seeds were placed in paper bags and left to dry and degas overnight at room temperature, before being stored at 4°C.

For the dry heat treatment, seeds from each subsample were placed in a 21.6 cm × 27.9 cm envelope that was placed flat on the shelf of a dryer set at 70°C for 5 d. All envelopes from the same block were placed on the same shelf. After the treatment, the envelopes were removed and stored at 4°C.

Vitaflo®-280 (carbathiin 0.514 mL a.i. kg^{-1} of seeds/thiram 0.437 mL a.i. kg^{-1}) was applied on seeds one subsample at a time. After treatment, seeds were stored at 4°C.

Effect of treatments on *F. graminearum* and *B. sorokiniana* contamination

To evaluate the effect of treatments on *F. graminearum* contamination, 50 seeds per subsample were plated onto potato dextrose agar medium (PDA Difco) (seven to eight seeds per Petri dish). Dishes were placed under fluorescent light (23 cm away from the light), with a photoperiod of 16 h at room temperature (20-22°C) for 7 d. After the incubation period, seeds showing *Fusarium* mycelia were counted and an agar plug (0.5 cm diam) containing actively growing mycelia was transferred onto sucrose nutrient agar (SNA; Nirenberg 1981) to identify *F. graminearum* based on

Table 1. Characteristics of the barley and wheat seed lots used in the study.

Seed lot (named by cultivar)	Type	Year of harvest	Provider or location
Barley			
Nd (name not provided)	six-row	2009	La Coop Fédérée
Newdale1	two-row malting	2009	Semican
Tradition	six-row malting	2010	Semican
Newdale2	two-row malting	2009	Semican
AC Hawkeye	six-row hulless feed	2011	Semican
Newdale3	two-row malting	2011	Semican
Wheat			
AC Barrie	HRS[a]	2007	St-Mathieu-de-Beloeil
AC Brio1	HRS	2007	Lévis
AC Brio2	HRS	2007	St-Mathieu-de-Beloeil
Orléans1	HRS	2007	Lévis
Torka	HRS	2011	St-Augustin-de-Desmaures
Orléans2	HRS	2008	Lévis

[a] HRS = Hard red spring wheat.

the descriptions by Nelson *et al.* (1983) and, consequently, to determine the number of seeds infected by *F. graminearum*. To evaluate *B. sorokiniana* contamination rates, 50 additional seeds per subsample were plated onto PDA-benomyl medium as described by Pouleur and Couture (2013). After 7 d of incubation in darkness at room temperature, the number of seeds showing typical *B. sorokiniana* dark green mycelia and conidia were directly counted.

Effect of treatments on germination, emergence, root rot index and seedling biomass

One hundred seeds per subsample of each treatment were planted in a sand substrate in five seed trays (32.4 cm wide × 52.7 cm long × 7.9 cm deep), with 20 seeds per tray. Each tray contained five rows of 20 seeds, each row corresponding to the five treatments per block per lot. The experimental unit was the 100 seeds planted in the five trays. The trays were placed in a growth cabinet in which temperature was maintained at 25°C, with 75% RH, a 13,000 lux light intensity, and a 16 h photoperiod. Trays were watered as needed during the first few days, then every day thereafter. Fertilization consisted in applying a solution of 2.5 g L^{-1} of 20-20-20 (Plant-Prod, Brampton, ON) in tap water 7 d, 10 d and 16 d after planting. Germination rate was estimated 7 d after seeding and, 14 d later, seedling emergence, root rot index and yield (aerial parts) of the 21-d-old seedlings were assessed. For assessing root rot, each seedling emerging from the 100 seeds planted was rated according to the 0-9 Horsfall-Barratt scale (Horsfall and Barratt 1945), and a root rot index was calculated using the following formula: Σ (class x number of plants in class) / total number of seedlings assessed. The aerial parts of the seedlings were placed in separate paper bags for each treatment combination and replicate, and dried at 70°C for 48 h before being weighed.

Effect of treatments on seedling emergence and yield parameters under field conditions

Each year, both the barley and wheat seed lots were tested in separate experiments using a randomized complete block design with four blocks. Experiments were conducted using organic fertilizer and mechanical weed control. They were conducted in the province of Quebec, Canada, at two locations: at the Centre de recherche sur les grains (CÉROM), Saint-Mathieu-de-Beloeil (45°34′N; 73°12′W), on heavy clays of the Saint-Urbain series (Humaquept), and at the Agronomy Research Station of Université Laval, Saint-Augustin-de-Desmaures (46°45′N; 71°27′W), on sandy loams of the Tilly series (Haplorthod). The experimental unit in Saint-Mathieu consisted of five 6-m-long rows, with 18 cm between rows, and the one in Saint-Augustin consisted of four 5-m-long rows, also with a row spacing of 18 cm. Seeding rates were 375 seeds m^{-2} for barley and 425 seeds m^{-2} for wheat. In Saint-Mathieu, plots were planted on 13 May 2010, 25 May 2011, and 18 May 2012, and in Saint-Augustin they were planted on 19 May 2010, 12 May 2011, and 19 May 2012. A nitrogen contribution of 25 kg N ha^{-1} was provided by a previous soybean crop and was supplemented with dry granular poultry manure (Acti-Sol™; Acti-Sol Inc., Saint-Wenceslas, QC) applied before planting at 55 kg N ha^{-1} for barley and 75 kg N ha^{-1} for wheat. The manure contained 36.8 g P_2O_5 kg^{-1} and 30.2 g K_2O kg^{-1}. For weed control, a tillage operation with a tine harrow was carried out before seedling emergence at both locations, and once more in Saint-Augustin at the three-leaf stage (Zadoks stage 13; Zadoks *et al.* 1974). Grain was harvested at maturity (Zadoks stage 92; Zadoks *et al.* 1974) on 13 August 2010, 2 September 2011, and 16 August 2012 for barley in Saint-Mathieu, on 20 August 2010, 2 September 2011, and 18 August 2012 for wheat in Saint-Mathieu, on 11 August 2010, 13 August 2011, and 14 August 2012 for barley in Saint-Augustin, and on 19 August 2010, 24 August 2011, and 23 August 2012 for wheat in Saint-Augustin.

To determine seedling emergence, seedlings were counted along 1 m on three rows at the three-leaf stage. At maturity, plots were windrowed, harvested, and grain weight was measured to determine yield. Thousand-kernel weight and test weight were determined for each plot based on the methods described in the Official Grain Grading Guide (Canadian Grain Commission 2011).

Statistical analyses

Data from each seed lot were analyzed separately. For data collected in the laboratory and growth cabinet tests, an analysis of variance was carried out using the GLM procedure of SAS (SAS Institute Inc. 2010). For field experiments, the combined data of Saint-Mathieu and Saint-Augustin were subjected to an analysis of variance using PROC MIXED of SAS, in which seed treatments and locations were considered fixed effects and the four blocks were considered random effects. When significant ($P < 0.05$), treatment means were compared using Fisher's protected least significant difference (LSD) test.

RESULTS AND DISCUSSION

F. graminearum and *B. sorokiniana* contamination in seed lots

For all barley seed lots, both AAV treatments and dry heat reduced *F. graminearum* contamination when compared with the control, whereas Vitaflo®-280 had no effect (Fig. 1A). For the three lots highly contaminated with *F. graminearum* (Nd, Newdale1 and Tradition), AAV-L, AAV-H, and dry heat reduced contamination below the *Fusarium* spp. infection threshold of 15%, above which lots should be rejected as seed (rejection threshold) according to Nielsen (2000). In the present study, we used a rejection threshold of 15% for *F. graminearum*. AAV-H also reduced the level of *B. sorokiniana* contamination in five barley lots, and AAV-L did so in three barley lots, whereas dry heat was not effective (Fig. 1B). In barley, Vitaflo®-280 decreased the level of contamination under the rejection threshold of 30% in two of the four lots highly (> 50%) contaminated by *B. sorokiniana* (Newdale2 and AC Hawkeye; Fig. 1B). Among those highly contaminated seed lots, AAV-H reduced the level of infection below the threshold for AC Hawkeye only, while AAV-L and dry heat did not do so in any of the seed lots.

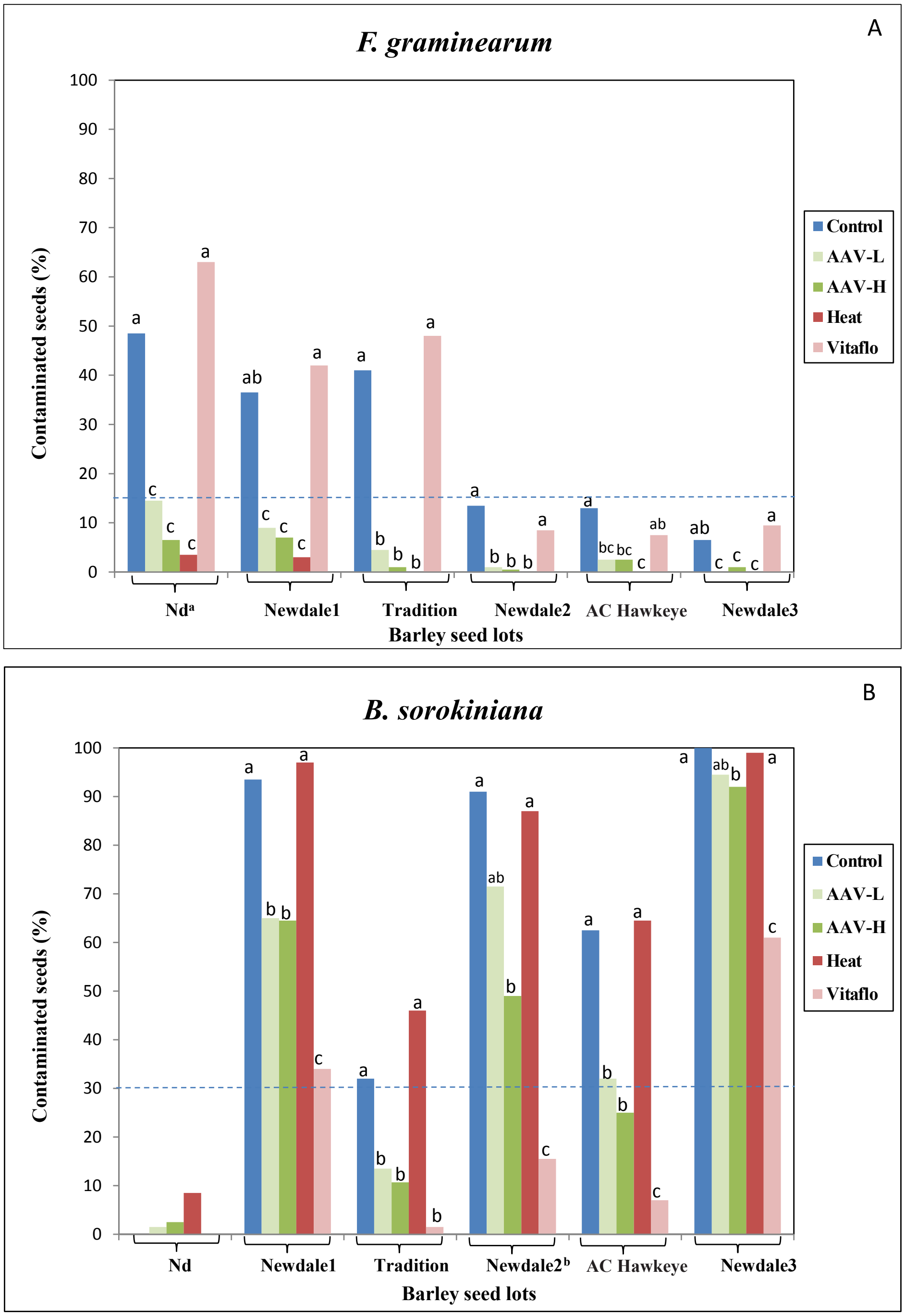

Fig. 1. Effect of seed treatments on seed contamination by *F. graminearum* (A) and *B. sorokiniana* (B) in six barley seed lots. For each lot, columns headed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$). The dashed line represents the threshold above which seed lots should be rejected as seed according to Nielsen (2000). [a] Nd = not determined; the name of the cultivar was not provided. [b] For this lot and this variable, means were log detransformed.

In wheat, all treatments, including Vitaflo®-280, reduced *F. graminearum* contamination to levels below the rejection threshold of 15% in three of the highly contaminated seed lots (AC Barrie, AC Brio2 and Torka; Fig. 2A), with the exception of Vitaflo®-280 in the AC Barrie seed lot. For *B. sorokiniana*, AAV-H significantly decreased its detection in four of the six wheat lots, and AAV-L and dry heat did so in two seed lots (Fig. 2B).

For barley, Vitaflo®-280 was generally less effective at reducing *F. graminearum* contamination compared with *B. sorokiniana*. The lack of efficacy of Vitaflo®-280 against seed-borne *F. graminearum* was in agreement with previous research conducted on winter wheat (Duthie and Hall 1985). In contrast, Vitaflo®-280 was found to decrease the level of *B. sorokiniana* contamination under the rejection threshold of 30% for all highly contaminated

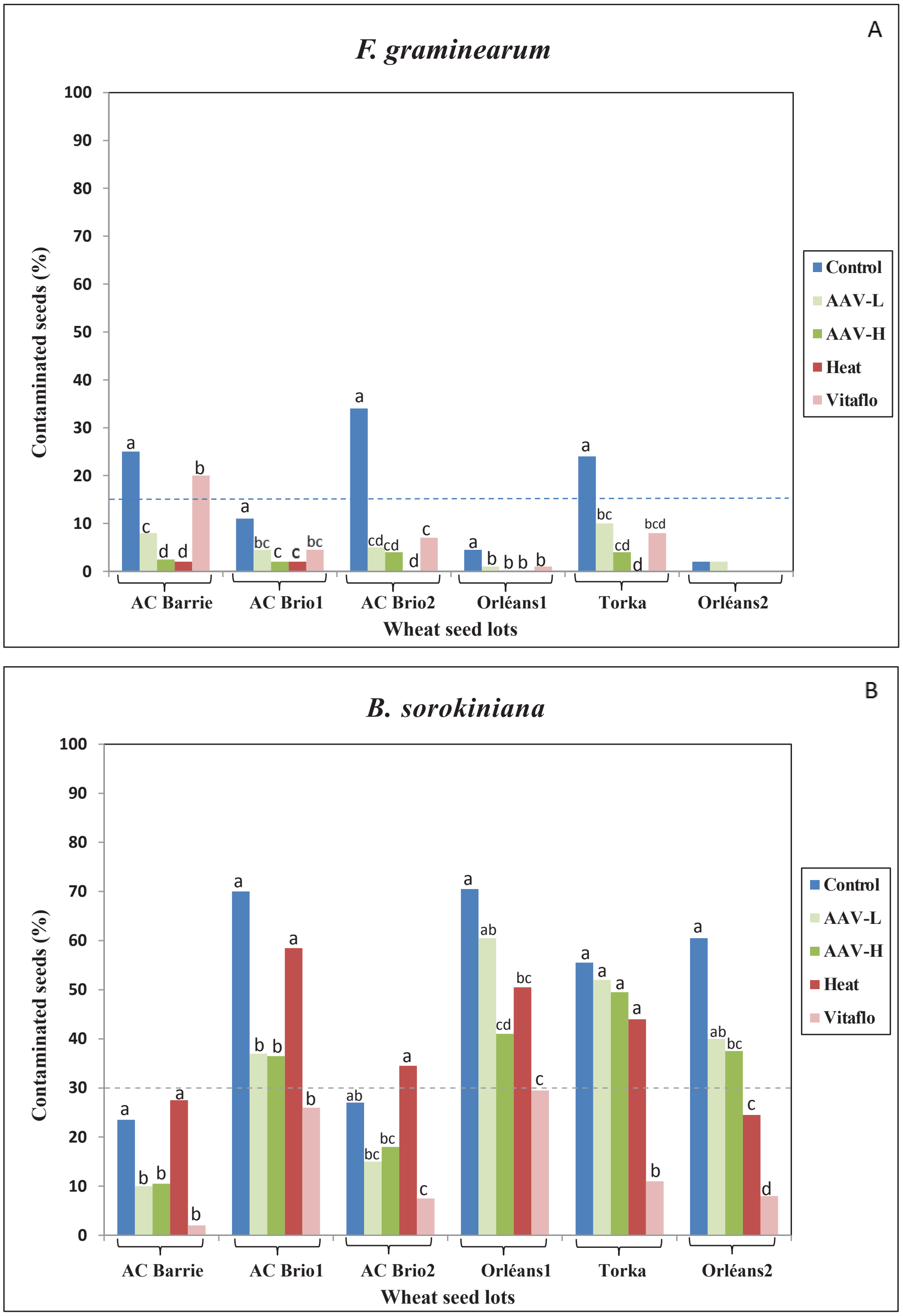

Fig. 2. Effect of seed treatments on seed contamination by *F. graminearum* (A) and *B. sorokiniana* (B) in six wheat seed lots. For each lot, columns headed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$). The dashed line represents the threshold above which seed lots should be rejected as seed according to Nielsen (2000).

wheat lots (AC Brio1, Orléans1, Torka, and Orléans2; Fig. 2B), whereas dry heat could only do so in one lot (Orléans2), and none of the AAV treatments had any effect on any of the seed lots.

For organic barley and wheat production, AAV-H seems to be the most promising treatment because it could reduce both pathogens, whereas dry heat was effective for the control of seed-borne *F. graminearum*, but had little to no effect on *B. sorokiniana* in wheat and barley lots, respectively. The efficacy of dry heat in controlling *F. graminearum* in wheat and barley seeds is in accordance with the results reported by Clear *et al.* (2002). The tolerance of *B. sorokiniana* in cereal seeds heated at 70°C for a few days was also observed in previous studies (Couture and Sutton 1980; Clear *et al.* 2002). The difference between *B. sorokiniana* and *F. graminearum* in their tolerance to heat treatment or desiccation might be due to differences in their cell components, cell wall or other parts of mycelial cells or spores.

Germination, emergence, root rot index and seedling biomass

For barley and wheat, the AAV-L, dry heat and Vitaflo®-280 treatments either increased seed germination or had no effect when compared with the control (Tables 2 and 3). Seed germination was improved by AAV-H in one barley lot (Newdale2; Table 2), but was reduced by this treatment in three wheat lots (AC Brio1, Orléans1 and Orléans2; Table 3) and in the AC Hawkeye hulless barley seed lot (Table 2); the four latter lots (AC Brio1, Orléans1, Orléans2 and AC Hawkeye) were highly contaminated with *B. sorokiniana* (Tables 2 and 3). Furthermore, in a wheat seed lot slightly contaminated with *F. graminearum* (11%) and *B. sorokiniana* (18%) (data not shown), germination was also reduced by AAV-H. Thus, AAV-H may damage embryos to such an extent as to reduce seed germination in hulless species like wheat or hulless barley cultivars such as AC Hawkeye. In previous studies (Pouleur *et al.* 2008), AAV-L had no effect on seed germination. A higher dose was tested in the present study with the objective of improving the efficacy of AAV in controlling *B. sorokiniana* in seeds. Emergence was assessed 14 d later and was lower in AAV-H than in the control for two (AC Brio1 and Orléans2; Table 3) of the four seed lots where germination was affected by AAV-H. In addition, there was no difference in aboveground leaf biomass between the AAV-H treatment and the control in three of those four seed lots (AC Hawkeye, Orléans1, Orléans2; no data available for AC Brio1). Perhaps the surviving seedlings compensated for those that did not emerge.

For barley (Table 2), except for AC Hawkeye, AAV-H, dry heat and Vitaflo®-280 had higher emergence and leaf yield than the control, while similar trends were observed with AAV-L in seed lots highly contaminated with *F. graminearum* (Nd, Newdale1, and Tradition). For AC Hawkeye, there were no differences between treatments for leaf yield, and only Vitaflo®-280 increased emergence whereas the other treatments were not different from the control. In wheat (Table 3), emergence and leaf yield of seed lots highly contaminated with *F. graminearum* (AC Barrie [emergence only], AC Brio2, and Torka) increased under all treatments, except for AAV-H and Torka where there was no effect. For the other seed lots (AC Brio1, Orléans1, and Orléans2), treatments either improved, reduced (AAV-H), or had no effect on emergence and leaf yield. Vitaflo®-280 increased leaf yield in three out of four barley lots (Table 2), and in three out of four wheat lots (Table 3), while AAV-H increased leaf yield in three barley lots and one wheat lot. Finally, dry heat increased leaf yield in three barley and two wheat lots, while AAV-L did so in one barley and two wheat lots.

Root rot index was lower or remained unchanged under seed treatment as compared with the control (Tables 2 and 3). Treatment with AAV-H or Vitaflo®-280 reduced root rot index in four barley and four wheat lots, while AAV-L and dry heat reduced root rot index in three barley and three wheat lots. AAV-H was more efficient than Vitaflo®-280 at controlling root rot in three barley lots and two wheat lots, while AAV-L and dry heat were more efficient than Vitaflo®-280, but only in one barley seed lot (Nd lot; Table 2).

For barley, emergence and root rot index results illustrated a significant impact of seed treatments compared with the control treatment for seed lots infected by *F. graminearum*, whereas seed treatments generally appeared less efficient for seed lots infected by *B. sorokiniana*. According to the laboratory and cabinet trials, AAV-H seems to have the best potential to improve phytosanitary seed quality and, consequently, to reduce root rot, but it may have deleterious effects on the embryos of hulless cereals.

Seedling emergence and yield parameters

In the field experiments, emergence was either increased, unchanged, or decreased by the treatments (Tables 4 and 5). AAV-H had a negative effect on emergence in the barley and wheat seed lots in which it reduced germination (AC Hawkeye (Table 4); AC Brio1, Orléans1, and Orléans2 (Table 5)), and also in the Nd barley seed lot (Table 4). For AC Hawkeye, all non-chemical treatments had a negative effect on emergence, while Vitaflo®-280 enhanced this variable compared with the control. In the Newdale seed lots, treatment responses varied (Table 4). For example, compared with the control, AAV-L, AAV-H, and Vitaflo®-280 significantly increased emergence in Newdale1, while dry heat and Vitaflo®-280 increased emergence in Newdale2. In the barley seed lot Tradition (Table 4) and in the wheat lot AC Barrie (Table 5), treatments had no effect on emergence as compared with the control. In general, dry heat, Vitaflo®-280 and AAV-L increased emergence in the other wheat lots (Table 4); however, with AAV-L, this increase was not significant in AC Brio1 and Orléans2, and a similar trend was observed with Vitaflo®-280 in AC Brio 2.

In contrast to leaf yield responses assessed in the growth cabinets, seed treatments had no significant positive effects on grain yield in any of the twelve lots tested (Tables 4 and 5). Better efficacy of seed treatments under controlled versus field conditions has been reported previously (Celetti and Hall 1987; Mihuta-Grimm and Forster 1989). In the present study, the drier conditions deliberately used in the growth cabinets with a sand substrate that promotes root diseases may explain the differences observed between the two environments (in the cabinets and in the field). Fernandez *et al.* (2010) also observed that seed treatments were less effective at reducing root discoloration caused by seed-borne *Fusarium* spp. and *B. sorokiniana* in a year when precipitation and mean temperature in late spring and early summer were higher than in the other years of the study. Also, in our field experiments, other soil-borne pathogens that were not present in the sand substrate may have interfered and caused root diseases and, consequently, masked the beneficial effect of the seed treatments. AAV-H, which affected germination in three wheat seed lots (Table 3) and in the hulless barley seed lot (Table 2), also decreased grain yield in two of these seed lots (AC Brio1 and Orléans2; Table 5), for an average yield reduction of 9%.

Table 2. Effect of seed treatments on germination in six barley seed lots, and other parameters assessed on 21-d-old seedlings grown in cabinets.

Treatment	Nd[a] (49-0)[c]	Newdale1 (37-94)[c]	Tradition (41-32)[c]	Newdale2 (14-91)[c]	AC Hawkeye[b] (13-63)[c]	Newdale3 (7-100)[c]
Germination (%)						
Control	74.5	82.8	78.5	90.8 bc	70.5 b	77.3
AAV-L	77.5	90.3	83.0	91.8 abc	73.0 b	80.3
AAV-H	73.0	81.0	82.8	95.8 a	59.8 c	85.8
Heat	69.5	80.8	83.8	93.3 ab	73.8 b	86.3
Vitaflo®-280	79.3	89.3	79.0	93.0 ab	83.3 a	86.5
Emergence (%)						
Control	26.5 b[d]	58.5 b	44.5 b	54.3 d	54.8 bc	51.0 c
AAV-L	68.0 a	81.3 a	69.3 a	59.8 bcd	53.5 bc	54.5 bc
AAV-H	69.0 a	77.8 a	77.8 a	67.3 ab	49.5 c	61.5 ab
Heat	66.3 a	67.3 ab	70.0 a	63.3 bc	58.3 bc	67.5 a
Vitaflo®-280	33.3 b	80.8 a	67.5 a	72.3 a	74.5 a	68.5 a
Root rot index (0-9)						
Control	7.4 a	5.8 a	5.6 a	6.4 a	5.8	7.0 a
AAV-L	3.2 b	4.0 c	3.7 b	5.9 a	5.0	7.0 a
AAV-H	1.7 c	3.1 d	2.7 c	5.2 b	5.0	7.0 a
Heat	2.0 c	5.2 ab	4.4 b	6.2 a	5.3	6.1 b
Vitaflo®-280	7.2 a	4.3 bc	4.0 b	4.8 b	4.5	6.4 b
Leaf yield (g DM)						
Control	–	–	1.90 b	2.05 c	1.76	1.54 c
AAV-L	–	–	2.70 a	2.40 bc	1.63	1.82 abc
AAV-H	–	–	2.92 a	3.14 a	1.39	1.97 ab
Heat	–	–	2.90 a	2.63 b	1.63	2.03 a
Vitaflo®-280	–	–	2.74 a	3.56 a	2.08	1.87 ab

[a] Nd = not determined; the name of the cultivar was not provided.

[b] Hulless cultivar.

[c] Initial seed contamination by *F. graminearum* (control; Fig. 1A) and *B. sorokiniana* (control; Fig. 1B), respectively.

[d] Within each column, means followed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$).

Table 3. Effect of seed treatments on germination in six wheat seed lots, and other parameters assessed on 21-d-old seedlings grown in cabinets.

Treatment	AC Barrie (25-24)[a]	AC Brio1 (11-70)[a]	AC Brio2 (34-27)[a]	Orléans1 (5-71)[a]	Torka (24-56)[a]	Orléans2 (2-61)[a]
Germination (%)						
Control	80.8 bc	80.5 b	82.5	79.0 cd	66.5 bc	71.5 bc
AAV-L	87.0 ab	78.5 b	85.0	82.3 bc	76.3 b	73.3 bc
AAV-H	79.5 c	62.3 c	84.0	68.0 e	64.5 c	58.0 d
Heat	84.8 abc	89.5 a	88.3	85.3 b	90.5 a	78.3 ab
Vitaflo®-280	89.8 a	94.5 a	88.0	95.3 a	91.5 a	85.0 a
Emergence (%)						
Control	69.3 c[b]	70.8 b	54.3 b	49.6 c	57.8 c	55.8 b
AAV-L	85.5 a	74.8 b	69.0 a	56.9 bc	68.8 b	59.5 b
AAV-H	78.5 ab	60.3 c	68.3 a	47.7 c	60.5 bc	48.5 c
Heat	80.8 ab	87.0 a	71.3 a	61.2 b	87.3 a	60.8 b
Vitaflo®-280	83.8 a	91.0 a	72.3 a	70.9 a	87.3 a	72.3 a
Root rot index (0-9)						
Control	5.0 a	4.1 a	5.4 a	5.7 a	4.6 a	5.7
AAV-L	3.4 bc	3.4 bc	4.1 b	5.4 ab	4.0 ab	5.1
AAV-H	2.8 c	3.0 c	3.9 b	5.1 bc	3.9 ab	5.0
Heat	3.7 b	3.9 ab	4.1 b	5.3 ab	3.1 b	5.4
Vitaflo®-280	3.8 b	3.8 ab	4.0 b	4.8 c	3.2 b	4.9
Leaf yield (g DM)						
Control	–	–	3.13 c	1.83 ab	1.35 c	1.15 b
AAV-L	–	–	3.93 a	2.07 a	1.59 b	1.23 b
AAV-H	–	–	3.97 a	1.65 bc	1.39 c	1.10 b
Heat	–	–	3.72 ab	2.14 a	2.03 a	1.24 b
Vitaflo®-280	–	–	3.86 a	2.00 ab	2.02 a	1.67 a

[a] Initial seed contamination by *F. graminearum* (control; Fig. 2A) and *B. sorokiniana* (control; Fig. 2B), respectively.

[b] Within each column, means followed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$).

Table 4. Effect of seed treatments on emergence and yield in six barley seed lots tested in the field at Saint-Mathieu-de-Beloeil and Saint-Augustin-de-Desmaures, QC, in 2010-2012.

	2010		**2011**		**2012**	
Treatment	**Nd[a] (49-0)[c]**	**Newdale1 (37-94)[c]**	**Tradition (41-32)[c]**	**Newdale2 (14-91)[c]**	**AC Hawkeye[b] (13-63)[c]**	**Newdale3 (7-100)[c]**
Emergence (# plants m^{-2})						
Control	284 a[d]	239 c	231	232 c	239 b	210 b
AAV-L	311 a	308 a	243	261 bc	166 cd	330 a
AAV-H	247 b	279 ab	244	259 bc	135 d	324 a
Heat	288 a	274 abc	260	283 ab	171 c	325 a
Vitaflo®-280	289 a	303 a	258	309 a	288 a	346 a
Grain yield (kg ha^{-1})						
Control	5639	4065	4885	4271	3980	3806
AAV-L	5464	4041	4795	4363	4030	3570
AAV-H	5366	3974	5253	4282	3797	3889
Heat	5363	4072	4974	4381	3635	3735
Vitaflo®-280	5350	4031	4874	4425	4399	4258
1000-kernel weight (g 1000 $kernel^{-1}$)						
Control	40.6 a	36.1	39.0	41.5	37.8	38.9
AAV-L	39.6 b	35.8	38.7	41.3	38.3	37.1
AAV-H	40.8 a	35.9	39.5	41.4	39.1	37.6
Heat	40.2 ab	36.7	38.9	41.5	37.3	38.0
Vitaflo®-280	40.3 ab	36.6	38.9	41.1	38.2	38.4
Test weight (kg hL^{-1})						
Control	60.7	60.6	39.0	41.5	74.1 a	61.9
AAV-L	60.5	60.4	38.7	41.3	70.8 b	61.7
AAV-H	60.7	60.7	39.5	41.4	70.1 b	62.2
Heat	60.4	60.9	38.9	41.5	71.5 b	62.2
Vitaflo®-280	60.1	60.7	38.9	41.1	74.9 a	62.4

[a] Nd = not determined; the name of the cultivar was not provided.

[b] Hulless cultivar.

[c] Initial seed contamination by *F. graminearum* (control; Fig. 1A) and *B. sorokiniana* (control; Fig. 1B), respectively.

[d] Within each column, means followed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$).

Table 5. Effect of seed treatments on emergence and yield in six wheat seed lots tested in the field at Saint-Mathieu-de-Beloeil and Saint-Augustin-de-Desmaures, QC, in 2010-2012.

	2010		**2011**		**2012**	
Treatment	**AC Barrie (25-24)[a]**	**AC Brio1 (11-70)[a]**	**AC Brio2 (34-27)[a]**	**Orléans1 (5-71)[a]**	**Torka (24-56)[a]**	**Orléans2 (2-61)[a]**
Emergence (# plants m^{-2})						
Control	361 ab[b]	279 b	223 cd	166 c	310 bc	306 b
AAV-L	383 a	314 b	257 ab	222 b	371 a	330 b
AAV-H	318 b	311 c	205 d	98 d	251 c	206 c
Heat	394 a	368 a	283 a	249 ab	427 a	397 a
Vitaflo®-280	389 a	361 a	243 bc	269 a	418 a	407 a
Grain yield (kg ha^{-1})						
Control	3848	3379 a	2908	2355	2422	3413 a
AAV-L	3591	3334 a	2761	2499	2292	3467 a
AAV-H	3515	2994 b	2566	1715	2167	3161 b
Heat	3625	3518 a	2755	2554	2123	3532 a
Vitaflo®-280	3676	3543 a	2845	2775	2823	3612 a
1000-kernel weight (g 1000 $kernel^{-1}$)						
Control	31.9	32.8	34.5	33.5 b	30.2	37.3
AAV-L	32.1	33.6	34.8	34.3 ab	29.7	37.1
AAV-H	31.8	33.1	34.2	33.5 b	29.0	37.1
Heat	32.0	33.0	34.3	34.5 a	28.6	37.2
Vitaflo®-280	31.8	33.2	34.8	34.3 ab	31.2	37.2
Test weight (kg hL^{-1})						
Control	76.9	76.8	75.1	73.9	72.4	77.0
AAV-L	77.4	76.6	75.1	74.4	71.2	77.2
AAV-H	77.2	76.9	74.9	73.5	71.1	76.8
Heat	77.0	77.1	75.1	74.7	71.3	77.3
Vitaflo®-280	77.1	76.8	74.9	74.5	73.4	77.4

[a] Initial seed contamination by *F. graminearum* (control; Fig. 2A) and *B. sorokiniana* (control; Fig. 2B), respectively.

[b] Within each column, means followed by the same letter were not significantly different at $P \leq 0.05$ following a significant ANOVA test ($P < 0.05$).

In general, there were no treatment effects on 1000-kernel weight, except for a negative effect of AAV-L in the Nd barley seed lot (Table 4), and a positive effect of dry heat in the Orléans1 wheat seed lot (Table 5). Similarly there was no effect of treatments on test weight, except for a negative effect of AAV-L, AAV-H, and dry heat in the AC Hawkeye barley seed lot (Table 4).

Results from the present study show that it is more difficult to control *B. sorokiniana* than *F. graminearum* in cereal seed. All non-chemical treatments could reduce *F. graminearum*, whereas Vitaflo®-280 was less effective, especially in barley. Dry heat appears to be a relatively easy method to use for cereal seed disinfection, and it has been routinely used since 2000 for the treatment of cereal seed lots by the Seed Increase Unit at the Indian Head Research Farm, Agriculture and Agri-Food Canada, Indian Head, SK (Agriculture and Agri-Food Canada 2011; Gehl 2007). However, for the majority of the lots tested in the present study, and based on the results of previous studies (Clear *et al.* 2002), the dry heat treatment has no effect on *B. sorokiniana*. For reducing the impact of *B. sorokiniana*, Vitaflo®-280 was the most effective treatment. Among the non-chemical treatments, AAV-H decreased the detection of *B. sorokiniana* in the greatest number of seed lots tested but this reduction, except for one barley lot, was not below the rejection threshold of 30% for seed lots highly contaminated with this pathogen. Moreover, AAV-H should not be used for hulless types of cereals since it could reduce their germination. Further research is needed in order to find treatment methods that are both acceptable for organic farming and effective at controlling *F. graminearum* and *B. sorokiniana* in wheat and barley.

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