

## CORRELATING FIELD AND CONTROLLED-ENVIRONMENT STUDIES OF PINK SNOW MOLD RESISTANCE IN PACIFIC NORTHWEST, USA, GREENS-TYPE *POA ANNUA*

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### ABSTRACT

Plant breeders are developing “greens-type” *P. annua* L. cultivars. Their success will be limited in northern climates unless cultivars are pink snow mold (*Microdochium nivale* (Fr.) Samuels and I. C. Hallett) resistant. Field evaluation for snow mold resistance has proven troublesome; therefore, controlled-environment evaluations could assist plant breeders in identifying snow mold-resistant *P. annua* germplasm. The objective of this study was to determine the validity of using a controlled-environment chamber (snow mold chamber) to evaluate pink snow mold resistance in greens-type *P. annua* f. *reptans* (Hauskn.) T. Koyama collected in the Pacific Northwest, USA. Field and snow mold chamber evaluations for pink snow mold resistance were performed on 36 accessions of greens-type *P. annua* collected in Washington, Idaho, and Oregon (representing six Pacific Northwest, USA, eco-regions) and three bentgrass controls (‘L-93’ creeping bentgrass (*Agrostis stolonifera* L.), ‘SR-7200’ velvet bentgrass (*A. canina* L.), and ‘Tiger’ colonial bentgrass (*A. capillaris* L.)). Following field and chamber evaluations, the *P. annua* accessions, eco-regions of accession origin, and bentgrass controls were ranked based upon pink snow mold resistance. The field and snow mold chamber evaluation ranks were not correlated based upon accessions, accession eco-regions of origin, or controls; therefore, controlled-environment evaluation for pink snow mold resistance in Pacific Northwest *P. annua* germplasm should supplement, not substitute for, field evaluation.

### Abbreviations

GTPA, Greens-type *Poa annua*; IDR, Initial disease rating; PSM, Pink snow mold; RDR, Rate of disease recovery; WSUTRA, Washington State University Turfgrass Research Area

### Keywords

Annual bluegrass; Annual meadowgrass; *Fusarium*; *Microdochium nivale*; Snow mold chamber

### INTRODUCTION

Annual bluegrass has become a major turfgrass species on golf courses in the Pacific Northwest, USA; however, it is a weed in golf greens due to its lack of environmental and disease tolerances (Dernoeden, 1999; Perkins, 1974). Furthermore, annual bluegrass is difficult, if not impossible, to eliminate from creeping bentgrass golf greens in the Pacific Northwest, USA (Goss, 1978). Since many turfgrass managers cannot afford the measures required to eradicate annual bluegrass, they are forced to manage the species (Christians, 1998).

Over time, annual bluegrass golf greens usually evolve to become mostly, if not entirely, *P. annua* f. *reptans*, which has many characteristics that make it desirable for use on golf greens. Huff (1996) labeled the desirable form *reptans*, “greens-type” *P. annua* (GTPA). Yet, annual

bluegrass golf greens are usually a patchwork of various GTPA strains, all of which respond differently to management practices (Goss, 1978). Thus, a need exists to develop GTPA cultivars. Huff (1996) and White (1999) have both worked extensively with *P. annua* f. *reptans*. White released a cultivar that has been marketed as ‘Peterson’s Creeping Bluegrass’ and ‘True-Putt Creeping Bluegrass.’

Since diseases are a major obstacle to maintaining a healthy annual bluegrass stand (Perkins, 1974), cultivars of GTPA should contain disease resistance. One of the most damaging diseases that affects *P. annua* is pink snow mold (PSM) caused by the fungal pathogen *M. nivale* (Dernoeden, 1999). As a result, it is important to evaluate (screen) GTPA breeding material for PSM resistance. Yet, PSM may develop irregularly in the field due to many environmental and management variables, e.g., soil drainage, thickness of thatch, fertility, soil pH, uneven snow accumulation and duration, landscape slope, shade, and air drainage (Toshikazu and Beard, 1997). Such incidences could be prevented and normal snow mold development enhanced if researchers performed resistance screenings in a controlled environment. Therefore, controlled-environment techniques to

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Table 1. Pacific Northwest eco-regions and number of accessions from each eco-region.

Eco-region†	Number of accessions (n)
Columbia Plateau	4
Northern Rockies	3
Eastern Cascade Slopes and Foothills	1
Puget Lowlands	6
Willamette Valley	14
Coast Range	8

†Eco-region designation (United States Environmental Protection Agency, 2004).

identify snow mold resistance are desirable (Gaudet and Chen, 1986).

Controlled-environment evaluations are most practical and beneficial to plant breeders when they accurately represent similar field evaluations. Ricard's (2003) controlled-environment chamber evaluation of GTPA for PSM resistance would prove most useful if it correlated to a field evaluation. This study compared field and controlled-environment screening of Pacific Northwest GTPA for PSM resistance to determine if results were correlated based upon *P. annua* accession and accession eco-region of origin.

## MATERIALS AND METHODS

### Greens-type *P. annua* Accessions

From 78 golf courses throughout Idaho, Oregon, and Washington, Poole (Poole, 2000; Poole et al., 2001) collected 200 GTPA accessions, which were distinguished in morphology and physiology. The number of accessions (n) that Poole collected from each Pacific Northwest eco-region (Omernik, 1987; United States Environmental Protection Agency, 2004), on which the eco-region mean was based, ranged from n = 1 to n = 14 (Table 1). In October 2000, 36 accessions from Poole's collection were selected for further study based on turfgrass quality and

seed production potential. Selected accessions were removed from a space-plant nursery at Pullman, WA and placed in a glasshouse for propagation and over-winter storage. For the duration of the study, vegetative propagation and inflorescence removal were performed to prevent recombination and ensure the genetic integrity of accessions.

In May 2001 and July 2001, respectively, 121, 56- x 28- x 5.8-cm-deep plastic trays (flats for the field experiment) with drain holes and 172, 7.6- x 7.6- x 8.5-cm-deep pots (for the snow mold chamber experiment) were filled with a growth media that was prepared by thoroughly mixing 1:1 by volume sand (Roach Construction, Genessee, ID) and greenhouse potting soil (Soil Conditioners, Zillah, WA). In May 2001, each accession was transplanted into three flats (replications). Approximately 120 individual plants, each of which consisted of one to three tillers with green leaves and roots, were planted into a flat with even spacing between plants. As a basis for comparison, three "industry-standard" bentgrass controls (L-93 creeping bentgrass, SR-7200 velvet bentgrass, and Tiger colonial bentgrass) were also evaluated for resistance to PSM. *Agrostis* control species were each seeded into three flats at 1 g of seed flat<sup>-1</sup>. In July 2001, each accession was transplanted into three pots (replications). Approximately nine individual plants, each of which consisted of one to three tillers with green leaves and roots, were planted into each pot with even spacing between plants. Additionally, three pots of each *Agrostis* control were seeded at 0.08 g of seed pot<sup>-1</sup>. Finally, 36 pots of True-Putt Creeping Bluegrass (Dawson Seed Co., Ltd., Surrey, BC, Canada) were seeded at 0.08 g of seed pot<sup>-1</sup> and placed into three flats (12 pots flat<sup>-1</sup>).

All flats and pots were maintained in a glasshouse at Pullman, WA at 24°C day/19°C night, with a 14-h day/10-h night cycle. No supplemental lighting was provided. Accessions were watered daily, fertilizers were applied as needed, and accessions were clipped twice per week at a height of approximately 1.3 cm using hand-held, 12-volt-electric grass shears (Ryobi Outdoor Products, Inc., Chandler, AR).

### Field Experiment

In May 2001, a field plot at the Washington State University Turfgrass Research Area (WSUTRA) at Pullman, WA was prepared for planting by rototilling, harrowing, and rolling. The soil was Palouse silt loam (Pachic Ultic Haploxerolls, fine silty, mixed mesic) with a pH of 6.6, 3.3% organic matter, 26 µg g<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 300 µg g<sup>-1</sup> K<sub>2</sub>O using the NaOAc extraction method (University of Idaho Analytical Sciences Laboratory, Moscow, ID).

The plot was planted using a Gandy 77.5-cm (wide) drop spreader. Four, 1.6- x 23.5-m strips were planted with SR7100 colonial bentgrass at 50.4 kg of seed ha<sup>-1</sup>. Each SR7100 strip was separated by an unplanted (bare) 0.6-m strip. In July 2001, the bare strips were planted with Tiger

colonial bentgrass at 100.8 g of seed ha<sup>-1</sup> using a Scotts 60.9-cm-wide drop spreader. After each planting, irrigation was supplied as needed to maintain moist soil until 10 d after seedling emergence.

The field plot was mowed every-other day during the growing seasons of 2001, 2002, and 2003. In late winter, at the beginning of the 2002 and 2003 snow mold evaluations, mowing heights were 12.5 and 4.6 mm, respectively.

Supplemental fertility was applied approximately once per month during the growing seasons. During 2001, the field plot received 88.2, 6.2, and 60.7 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively, while during 2002, the plot received 284.8, 5.4, and 147.1 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively. Granular fertilizers were applied using a Scotts SR-2000 walk-behind, rotary spreader. Soluble fertilizers were applied with an AGPRO Series-25 sprayer (AGPRO Marketing & Manufacturing Inc., Lewiston, ID) that delivered 407 L ha<sup>-1</sup> at 276 kPa.

In September 2001, plants maintained in the greenhouse were transplanted from flats into 56- x 28- x 6-cm-deep cavities where sod had been removed from the Tiger colonial bentgrass strips (blocks). Sand was used to bring accessions transplanted from flats level with the surrounding turf. The 36 accessions and three controls in each Tiger colonial bentgrass strip were the experimental units. A 25-cm border of undisturbed sod served as a buffer between experimental units. The experiment was randomized complete-block design with three replications.

The winter of 2001-2002, had 64 d of snow cover. Following snow melt on 18 Feb. 2002, each accession was overlaid with a 61- x 33-cm grid comprised of 6.45-cm<sup>2</sup> blocks segregated by highly visible white string. Digital photos of each accession were then taken using a COOLPIX 990 digital camera (Nikon USA, Melville, NY) mounted on a tripod 1.1 m above the turfgrass surface. Photos were again taken on 21 Mar., 19 Apr., and 21 May 2002. During the winter of 2002-2003, there were 7 d of snow cover. Digital photos of each accession were taken on 21 Feb., 28 Mar., 21 Apr., and 22 May 2003.

The digital images were transferred to a personal computer and then projected on a screen at 1.65 m (w) x 0.86 m (h) using a NEC Model 1045 Projector (NEC USA, Inc., Melville, NY). All photos were viewed using ADOBE Photoshop version 5.0 LE (Adobe Systems, Inc., San Jose, CA). To increase the visual uniformity of appearance between accessions and more easily distinguish diseased plant tissue, Photoshop's lighting effects filter was used to render all photos.

To evaluate each experimental unit for PSM, first the total number of grid blocks that each accession occupied was recorded. Then, PSM-infected blocks were counted, to serve as the numerator in determining percentage of accession area affected by snow mold. Blocks

in which disease was visibly evident in 50% or more of the block were considered infected.

In March 2003, after snow melt, samples were taken to confirm that *M. nivale* (Teleomorph, *Monographella nivalis* (Schaff.) Müller and von Arx) was indeed the pathogen infecting the experimental units. The pathogen was isolated from several infected plants taken from various locations at the field plot. After isolation and propagation in the lab on Difco potato dextrose agar (PDA), samples were viewed both macroscopically and microscopically (1000x) and compared with descriptions of *M. nivale* provided by Booth (1971) and Domsch et al. (1980). *M. nivale* was confirmed to be the pathogen present.

### Snow Mold Chamber Experiment

At snow melt in 2002, isolates of *M. nivale* were collected on 26 March from seven locations at the WSUTRA. As in the field experiment, after isolation and propagation in the lab on Difco PDA, samples were viewed both macroscopically and microscopically and compared with descriptions of *M. nivale* provided by Booth (1971) and Domsch et al. (1980) to confirm pathogen identity.

Explants from each location with symptoms of disease were surface-sterilized for three minutes in 10% bleach by volume, rinsed twice in deionized H<sub>2</sub>O, and blotted dry with sterile paper towels. Explants were then placed on two petri dishes (plates) (six explants plate<sup>-1</sup>) that contained PDA with penicillin and streptomycin ("pen/strep"). The pen/strep was prepared by adding 10 mL Penicillin-Streptomycin Solution (100x) (Sigma-Aldrich, Inc., St. Louis, MO) to 1 L of PDA. The 14 plates that contained explants were then placed in a Percival I-35 incubator (Percival Manufacturing Co., Inc., Boone, IA) equipped with standard fluorescent lights at 15°C day/12°C night, with a 15-h day/9-h night cycle.

Mycelia that grew from explants into the pen/strep were subcultured onto 14 new plates that contained ½-strength PDA on 1 April. The plates were then placed in the incubator at 8°C day/6°C night. On 9 April, three of the six standard fluorescent lights were removed from the incubator and replaced with blacklights to provide near-UV light and induce conidial formation.

On 27 April, based upon observations of mycelia growth rates and morphology, it was determined that the mycelia collected from different locations at the WSUTRA represented different isolates of *M. nivale*. Three of the isolates were selected based upon growth rate: one slow, one intermediate, and one rapid. The three selected strains were subcultured onto 10 plates each of ¼-strength Difco PDA and placed into the incubator.

Additionally, to ensure compatibility, all three strains were subcultured together on two PDA plates (replications) and placed into the incubator. After 14 d growth on PDA, compatibility of strains was visually confirmed on 12 May by examining cultures for the

presence of mycelia from all three strains growing intermingled.

A conidial suspension was prepared on 10 July using sterile water and the three strains of *M. nivale* that had been previously cultured. Equal amounts of conidia from each isolate, determined using a haemocytometer, were used to prepare a suspension of approximately  $1 \times 10^6$  conidia mL<sup>-1</sup> of sterile water (Diamond and Cooke, 1999).

On 17 June 2002, plants that had been maintained in 7.6- x 7.6- x 8.5-cm-deep pots were moved from the glasshouse to the snow mold chamber for acclimation. The chamber had three shelves (blocks). Each shelf was fitted with three daylength-controlled 60-watt Spot-Gro™ (OSRAM Sylvania Inc., St. Mary's, PA) incandescent bulbs mounted 34 cm above the turfgrass surface. Each shelf held one set of three plastic trays with drainage holes (flats) (13 pots flat<sup>-1</sup>) plus one flat (12 pots) of True-Putt *P. annua*. The 36 accessions and the three bentgrass controls in each set of flats were the experimental units, which were randomized within sets. Each set was removed as a unit for rating. The True-Putt *P. annua* plants were used for destructive sampling to determine the PSM-rating frequency. The experimental design was a randomized complete block with three replications.

Initially (17 June, Day 1 of experiment), the snow mold chamber was set at 12°C, for gradual plant acclimation, and daylength was 10 h to mimic typical early November conditions at Pullman, WA. Photoperiod was decreased daily to mimic the decreasing daylength that would occur at Pullman, WA from 1 November to 14 December. Chamber temperature was decreased by 2°C every three to four days until 2 July (Day 16), when the temperature was held at 2°C for the duration of simulated snow cover. On 23 June and 3 July, all plants were clipped at a height of approximately 1.3 cm.

Immediately following preparation of a *M. nivale* conidial suspension on 10 July (Day 24), experimental units (pots of GTPA and bentgrass controls) and True-Putt *P. annua* were sprayed (inoculated) with approximately 1 mL of conidial suspension using a 950-mL hand-held spray bottle. After inoculation, each experimental unit was placed into a designated flat. Three flats containing experimental units were then placed adjacent to each other, creating a set of experimental units. Water-absorbent, non-sterile cotton (DE Healthcare Products, Denver, PA) was then placed onto the surface of each of the three sets and secured with tape. After all experimental units were covered with cotton, tap water was utilized to wet the cotton until saturation. The sets were then placed into the controlled-environment chamber with one set placed on each of three shelves (blocks).

On 6 August (Day 51), one day after artificial snow cover removal, photoperiod in the chamber was increased from 8 h, 30 min. to 10 h, 17 min. to mimic daylength at Pullman, WA on 15 February when snow cover usually subsides. To mimic the typical increases in daylength and

average temperature that occur at Pullman, WA during the spring after snow cover subsides, an increasing chamber daylength was initiated on 3 August, while chamber temperatures were increased beginning on 6 August to 4°C. Subsequent increases in temperature were made: 11 August to 6°C, 15 August to 8°C, 19 August to 10°C, and 23 August to 12°C.

In late July, to determine the rating interval, two pots (subsamples) of True-Putt that had been designated for destructive sampling were removed daily from each shelf (block). The cotton cover was removed from the pots and the grass examined for PSM. The cotton cover was not removed from any of the GTPA accessions or control experimental units. After the initial disease assessment on 5 August, daily examinations of the pots containing True-Putt were continued until a significant increase in disease became evident. Thus, a 4-d-rating interval was established.

Beginning 5 August, every four days, digital photographs of each accession were taken using a COOLPIX 990 digital camera (Nikon USA, Melville, NY) that was mounted on a tripod to mitigate variation among photographs. Digital photographs were discontinued after two months, since it was felt that germplasm failing to recover from PSM within two months of snow melt would not be ultimately marketed as "snow mold resistant." Before taking each photograph, four accessions were overlaid with a grid comprised of 1.6-cm<sup>2</sup> blocks segregated by white string. Thus, each digital photograph was used to evaluate a group of four experimental units. After the initial digital imaging was completed, all experimental units were placed back into their sets and returned to the chamber without cotton covering. Digital images were transferred to a personal computer, projected on a screen, and ratings performed using the same techniques as previously described for the field experiment.

### Disease Assessment Techniques and Analysis

Three disease resistance assessment techniques were common to both the field and snow mold chamber experiments. Each technique was used to assess the resistance in each accession, control, and eco-region (mean resistance of all accessions collected from an eco-region).

Initial disease ratings (IDR) (percentage of the area of an accession or control affected by disease), after snow melt in the field or removal of artificial snow cover in the snow mold chamber, was used to determine resistance to PSM based upon the technique of Vargas et al. (1972). IDR ratings were compared using the ANOVA feature of the Statistical Analysis System software (SAS) (SAS Institute Inc., Cary, NC). To compare PSM resistance among eco-regions, the mean IDR for all accessions from each eco-region (eco-region mean IDR) was calculated and analyzed.

Table 2. Correlations between the same resistance assessment techniques when used in different (field and snow mold chamber) screenings based upon comparisons of greens-type *Poa annua* accessions and Pacific Northwest eco-regions.

Evaluation basis	Resistance assessment technique	Spearman rank correlation coefficient	<i>P</i> - values
Accessions			
	†IDR <sub>FIELD</sub> vs. ‡IDR <sub>CHAMBER</sub>	0.08	0.65
	§RDR <sub>FIELD</sub> vs. ¶RDR <sub>CHAMBER</sub>	0.04	0.79
	#AUCRC <sub>FIELD</sub> vs. ††AUCRC <sub>CHAMBER</sub>	-0.23	0.16
Eco-regions			
	IDR <sub>FIELD</sub> vs. IDR <sub>CHAMBER</sub>	0.22	0.58
	RDR <sub>FIELD</sub> vs. RDR <sub>CHAMBER</sub>	0.07	0.86
	AUCRC <sub>FIELD</sub> vs. AUCRC <sub>CHAMBER</sub>	0.22	0.58

†IDR<sub>FIELD</sub> = Initial disease ratings after snow cover melted from the field.

‡IDR<sub>CHAMBER</sub> = First rating after the removal of artificial snow cover.

§RDR<sub>FIELD</sub> = Rate of disease recovery as slope of the best-fit line of percentage disease as a function of the time that plants were recovering from disease (not covered by snow).

¶RDR<sub>CHAMBER</sub> = Rate of disease recovery as slope of the best-fit line of percentage disease as a function of the time that plants were recovering from disease (not covered by simulated snow).

#AUCRC<sub>FIELD</sub> = Area under the crop recovery curve for the field evaluation of PSM resistance.

††AUCRC<sub>CHAMBER</sub> = Area under the crop recovery curve for the snow mold chamber evaluation of PSM resistance.

Rate of disease recovery (RDR) were based upon comparisons of the slopes of the best-fit lines of percentage of area affected by PSM disease as a function of time that plants were recovering from PSM disease (plants not covered by snow or simulated snow). Using SAS Proc GLM for repeated measures, it was determined through linear and quadratic contrasts that PSM disease decreased in a linear ( $P > 0.01$ ), rather than quadratic ( $P = 0.30$ ) manner. Therefore, probit transformation of percentage of PSM disease to fit a curve to a straight line (Large, 1942) was not used. Instead, using Excel 2000, best-fit lines for percentage of PSM disease as a function of time were calculated for each accession and bentgrass control using non-transformed data from all ratings. Excel also provided the calculation of slope for each line according to the equation  $y = mx + b$ . The eco-region mean slope was determined by averaging all accession slopes within each Pacific Northwest eco-region.

Potential of accessions and bentgrass controls to recover from PSM was assessed using the area under the crop recovery curve (AUCRC) (Lawton and Burpee, 1990). AUCRC was calculated using the equation:

$$\sum_{i=1}^{n-1} [(100 - X_{i+1} + 100 - X_i) / 2] (T_{i+1} - T_i); \text{ where } X$$

= percentage of necrotic foliage at observation  $i$  and  $T$  = time. To determine the relative ability of Pacific Northwest eco-regions to produce GTPA with a high potential to recover from PSM, the mean AUCRC for all

accessions from each eco-region (eco-region mean AUCRC) was calculated and analyzed.

For each PSM disease assessment technique, field and snow mold chamber accession and eco-region PSM-disease means were ranked using the Proc Rank feature of SAS. Spearman correlation coefficients for accession and eco-region ranks were then determined between the same parameters in the field and snow mold chamber using the SAS Proc Corr function.

## RESULTS

### Accessions

For each PSM-resistance assessment technique (IDR, RDR, or AUCRC), the Spearman-rank procedure indicated no significant ( $P < 0.05$ ) correlation between results of the field and the snow mold chamber for GTPA accessions, or controls (Table 2). Therefore, no similarity existed between the field and chamber assessments of the PSM disease resistance of Pacific Northwest GTPA accessions and controls at the accession level.

### Eco-regions

The Spearman-rank procedure indicated no significant correlation between eco-region results from the field and the snow mold chamber based upon any of the three PSM-resistance assessment techniques (Table 2). Thus, no similarity existed between the field and chamber assessments of PSM disease resistance in GTPA from any Pacific Northwest eco-regions.

## DISCUSSION

Bruehl et al. (1964) and Miedaner et al. (1993) both found correlation between field and controlled-environment assessments of resistance to *M. nivale* in winter wheat (*Triticum aestivum* L.) and winter rye (*Secale cereale* L.). However, Blomqvist and Jamalainen (1968) found that winter rye varieties differed in their genetic resistance to low-temperature parasitic fungi depending upon whether they were screened in the field, glasshouse, or laboratory. Such contrasting results suggest that experimental design, and particularly, environmental conditions may play a pivotal role in the expression of snow mold resistance. Indeed, Miedaner et al. (1993) concluded that the snow mold resistance of different rye genotypes was expressed differently under different environmental conditions.

Although measures were taken to provide similar conditions during all portions of this study, because of the countless variables involved in the production of controlled-environments (Dimock, 1967; McFarlane, 1978), it is extremely unlikely that every variable of the field environment was reproduced in the snow mold chamber. Therefore, as in previous studies (Jamalainen, 1974; de Kogel et al., 1997; Smeets and Wehner, 1997), cultivar x environment interactions could have prevented analogous field and snow mold chamber rankings in this study. Further research would be required to determine if environment x accession interactions affect PSM-resistance screenings of GTPA. Further experimentation might also reveal a snow mold chamber set-up that better mimics field conditions.

Research has indicated a biochemical influence of several peptides, proteins, and sugars on snow mold resistance in winter cereals (Ergon et al., 1998; García-Olmedo et al., 1998; Gaudet et al., 1999; Gaudet et al., 2000; Gaudet et al., 2001a; Gaudet et al., 2001b; Gaudet et al., 2003). Since PSM functions nearly the same in snow-covered wheat and annual bluegrass (Litschko and Burpee, 1987), it is assumed that these components may also affect the snow mold resistance in *P. annua* f. *reptans*. If such is the case, the combination of plant defense-related proteins and the 8-d-hardening period at 2°C for the snow mold chamber portion of this study could have affected the correlation results.

Gaudet et al. (2003) implicated various plant defense-related proteins in snow mold resistance. Moreover, in the most mature winter wheat plants, they found that PR-1a transcripts were insufficiently upregulated after one week of hardening. Thus, if PR-1a does play a role in PSM disease resistance of GTPA, the protein may not have been upregulated and able to function in the snow mold chamber portion of this study due to an insufficient hardening duration. Therefore, controlled-environment screenings of GTPA with longer hardening periods could help determine if hardening duration affects controlled-environment screenings of

GTPA. Additionally, further work to determine the function of defense-related proteins in the PSM disease resistance of GTPA could provide valuable information for determining a snow mold chamber screening protocol that would yield results similar to analogous field screenings.

## CONCLUSIONS

No correlations existed between field and snow mold chamber screenings based upon any resistance assessment technique at the accession or eco-region level. Therefore, the selection of GTPA germplasm to be eventually released as cultivars must be field-tested. Further research on PSM-resistance mechanisms and snow mold chamber set-up may yield controlled-environment assessment methods for GTPA that correlate to field trials.

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