An Artificial Inoculation Method to Select Mature Onion Bulbs Resistant to Fusarium Basal Rot

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Abstract. **Fusarium basal rot (FBR) of onion, which is caused by *Fusarium oxysporum* f.sp. *cepae* (Hanzawa) Snyder & Hansen (FOC) results in a substantial loss of marketable bulbs worldwide. One of the main reasons for the lack of FBR-resistant short-day cultivars is the unreliable screening methods available for the mature bulb stage when significant economic damage occurs. The objective of this study was to develop an artificial inoculation method with better quantification of inoculum for an effective selection of FBR-resistant mature onion bulbs. Mature bulbs of seven New Mexican short-day onion cultivars, along with susceptible and tolerant controls, were selected and evaluated for FBR resistance using mycelial and conidial inoculation methods, respectively. Transversely cut basal plates of mature bulbs were inoculated artificially with mycelia or conidia (12×10^5 spores/mL in 2014 and 3×10^5 spores/mL in 2015 embedded in potato dextrose agar plug) of a virulent FOC isolate 'CSC-515'. Mature bulb evaluation using a visual rating scale (1 = no disease; 9 = >70% basal plate infected) revealed a high degree of FBR severity and incidence irrespective of the genetic background of the cultivars, minimizing the chance of disease escape, which is a significant problem in field inoculation. An attempt to inoculate intact basal plates postharvest resulted in minimal disease development, suggesting that mechanical resistance was conferred by the dry outer layer of the basal plate. The high selection pressure conferred by the conidial inoculation method developed in this study can effectively screen FBR-resistant onion bulbs to replace an unreliable field screening. Concentrations of the conidia lower than 3×10^5 spores/mL are recommended to detect subtle genetic differences in FBR resistance among the onion cultivars and their selected population.**

Onion is an important vegetable commodity in the world market and accounts for 23.8% of the world's total vegetable production area (FAOSTAT, 2018). The United States is the third largest producer of dry onion bulbs in the world after China and India (FAOSTAT, 2018). Despite producing 39% of the summer nonstorage onion (NASS, 2017), onion production in New Mexico experiences substantial crop damage by FBR caused by FOC. FOC infects the roots and bulbs of onions grown in temperate and subtropical regions (Brayford, 1996). The 4-year crop rotations with nonhost crops (Sumner, 1995) and soil fumigation (Bacher et al., 1989) were found to be ineffective for controlling FBR due to the lack of suitable fields for rotation and high capital investment (Thornton and Mohan, 1996), respectively. Fumigation also resulted in the elimination of beneficial soil microorganisms (Brown, 2001; Jawson et al., 1993). Because of these limitations, the development of resistant cultivars is the best possible alternative (Sumner, 1995) because that will allow onion growers to use the same fields for multiple crops without the need for soil fumigation.

Unknown mechanisms of resistance along with a highly outcrossing nature (Taylor et al., 2019), inbreeding depression (McCallum et al., 2008; Taylor et al., 2019), and daylength specificity (Taylor et al., 2019) are some of the major challenges of developing FBR-resistant onion cultivars. A review of studies involving mostly long-day plant germplasm suggested that multiple modes of inheritance of single or multiple nuclear and cytoplasmic genes are interacting at different crop growth stages (Cramer, 2000; Marzu, 2015). A putative marker–trait association study confirmed quantitative trait loci for FBR resistance in a diverse set of germplasm involving short-day, intermediate-day, and long-day germplasms (Taylor et al., 2019). Genotyping using single-nucleotide polymorphism markers grouped the germplasms according to their daylength requirement, indicating different daylength-adapted gene pools (Taylor et al., 2019). Onion breeding programs are forced to develop locally adapted selection strategies worldwide as the exchange of breeding germplasm is restricted.

The most commonly practiced method of screening for FBR-resistant onion cultivars is seedling screening using a virulent FOC isolate or isolates (Caligiore-Gei et al., 2020; Esfahani et al., 2013; Galván et al., 2008; Kalman et al., 2020; Lopez and Cramer, 2004; Mandal et al., 2020; Özer et al., 2004; Rout et al., 2016; Taylor et al., 2013), followed by field screening that uses natural FOC isolates (Özer et al., 2004). Even though these two methods were used extensively to develop highly FBR-resistant commercial intermediate-day and long-day hybrids (Cramer, 2000; Goldman, 1996; Retig et al., 1970), limited success has been realized for short-day cultivars (Gutierrez and Cramer, 2005; Gutierrez et al., 2006; Lopez and Cramer, 2004). The main reasons for this lack of success are due to the random chance of host–pathogen interactions during field inoculation (Mandal et al., 2020), a complicated cultivar–isolate relationship (Saxena and Cramer, 2009), and isolate virulence differences among geographical regions (Caligiore-Gei et al., 2020; Sasaki et al., 2015). Even though FBR resistance at the seedling stage could be translated into the later growing stages (Taylor et al., 2019), the seedling resistance may not be useful during storage of a dormant bulb (Mandal et al., 2020) for which environmental conditions are more favorable for FOC damage (Cramer, 2000). A resistance response against FOC in the mature bulb is characterized by slowing of disease progression (Cramer, 2000) due to lower pectic enzyme production (Holz and Knox-Davies, 1985a, 1985b) and/or the production of antifungal compounds (Özer et al., 2003, 2004). Therefore, FBR-resistant mature bulbs could be screened during storage after artificial inoculation.

The mature bulb stage has never been used to develop FBR-resistant onion cultivars, nor is there any reliable bulb inoculation method. Therefore, the objective of this study was to develop an artificial inoculation method involving quantification of the inoculum for effective selection of FBR-resistant mature bulbs.

Materials and Methods

Plant materials. The present study was performed at the Fabian Garcia Science Center (FGSC) in Las Cruces, NM, from Oct. 2013 until July 2015. Seven autumn-sown and genetically diverse Grano-type onion cultivars, which differ in their time of maturity,

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Table 1. Name, year of release, description, and pedigree of short-day cultivars used to test artificial inoculation methods.

Name	Yr of release	Description	Origin	Reference
NuMex Camino	2003	Open-pollinated, Grano-type, fall seedling, early-maturing, single-centered, bolting-resistant, pink root-resistant, round globe, firm, yellow skin	'Excel986B' and 'TEG502PRRBR'	Cramer and Corgan, 2003a
NuMex Sweetpak	1999	Open-pollinated, Grano-type, fall seedling, early-maturing, single-centered, bolting-resistant, tolerant to pink root, nearly round, pale yellow skin	'Texas Grano 1015Y', 'Excel 986B', and 'Texas Grano 502 PRR'	Wall and Corgan, 1999
NuMex Chaco	2003	Open-pollinated, Grano-type, fall seedling, intermediate-maturing, single-centered, bolting-resistant, pink root-resistant, round top shape, firm, yellow skin	'TEG502PRRBR' and 'Southport White Globe'	Cramer and Corgan, 2001
NuMex Mesa	1995	Open-pollinated, Grano-type, fall seedling, intermediate-maturing, bolting-resistant, pink root-resistant, firm, yellow skin	'NuMex BR1' and 'Buffalo'	Corgan, 1996a
NuMex Crispy	1995	Open-pollinated, Grano-type, fall seedling, intermediate-maturing, bolting-resistant, pink root-resistant, firm, white skin	'Excel 986A' and 'Temprana'	Corgan, 1996b
NuMex Vado	1995	Open-pollinated, Grano-type, fall seedling, late-maturing, bolting-resistant, pink root-resistant, round-top shaped, firm, yellow skin	'NuMex BR1' and 'Ben Shemen' selection	Corgan, 1995
NuMex Luna	1995	Open-pollinated, Grano-type, fall seedling, maturing later than 'NuMex Vado', bolting-resistant, pink root-resistant, round top shape, firm, yellow skin	'NuMex BR1' and 'Ben Shemen' selection	Corgan, 1995

were used for this study (Table 1). These over-wintering cultivars are preferred by New Mexican onion growers due to their early maturity (Walker et al., 2009), lower FBR disease incidence (Lopez and Cramer, 2002, 2004), and resistance or tolerance to pink root disease (*Phoma terrestris* E.M. Hans.), which is positively correlated with FBR disease severity (Cramer, 2000). An FBR-tolerant control, 'Serrana' (Monsanto Vegetable Seeds, Woodland, CA), and a susceptible control, 'NuMex Crimson' (Cramer and Corgan, 2003b), were included in this study to ensure successful disease development and to compare cultivar susceptibility.

Field production of mature bulbs. Entries were grown in raised beds (5.5 m × 0.56 m) arranged in a randomized complete block design with four replications. Triple superphosphate (0N–45P–0K; Crop Production Services, Vado, NM) was applied at 170.10 kg·ha^{-1} during bed preparation. Seeds and transplants (for gap-filling only) were planted in two rows to maintain ≈110 plants per bed. The plant-to-plant distance was 10 cm after thinning at the stage with four to five vegetative leaves. Standard cultural practices for growing onions in southern New Mexico were used (Walker et al., 2009). Subsurface drip irrigation tape (T-Tape; T-Systems International, San Diego, CA) with 20-cm emitter distances was placed 10 cm below the surface and used to water (flow rate: 500 LPH/100-m length). Fish fertilizer (2N–3P–1K; Neptune's Harvest, Gloucester, MA) and acid-based liquid fertilizer (26N–0P–0K–6S; Western Blend, Inc., Las Cruces, NM) were supplied through the drip irrigation system according to the need of the crop and stopped 3 to 4 weeks before harvest. Bulbs were harvested when plant tops were 80% lodged.

Inoculation methods. Mycelial and conidial inoculation methods were used for the selection and evaluation of FBR resistance in mature bulbs, respectively. A long-term stored, highly virulent FOC isolate, 'CSC 515', was used for both types of inoculation methods because of its ability to efficiently separate FBR-susceptible and FBR-resistant individuals (Saxena and Cramer, 2009). For the mycelial inoculation method, a thin section (≈0.25–0.30 mm) of the basal plate tops containing the dead roots and dried outer tissue were removed to expose the inner tender tissue. Transversely cut basal plates were then smeared with a 0.5-cm^2 potato dextrose agar (PDA) plug containing 10-day-old actively growing mycelia.

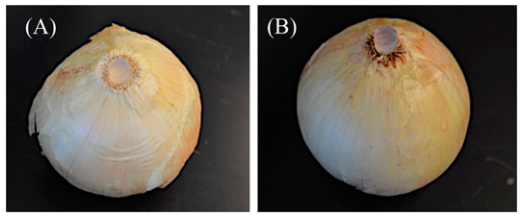

Fig. 1. Inoculum delivery via potato dextrose agar (PDA) (**A**) on a transversely cut basal plate and (**B**) on an intact basal plate.

For the conidial inoculation method, a spore suspension was prepared by first rinsing and scraping mycelial plugs on PDA plates with sterile distilled water. Multiple layers of cheesecloth were used to remove any mycelial fragments from the solution. The conidial suspension was mixed with PDA using a magnetic stirrer to evenly distribute spores for a final concentration of 12×10^5 spores/mL in 2014, which was reduced to 3×10^5 spores/mL in 2015. The mixing was performed just before the PDA started to solidify at a temperature of 35 °C, and the PDA spore suspension was immediately poured into petri plates. A few of these plates that were not used for inoculation were incubated in a growth chamber to confirm the viability of conidia through the production of excessive fungal growth. Plates were prepared the night before an inoculation event to prevent spore concentration changes and mycelium development. Intact 1-cm-diameter plugs from these plates were placed on transversely cut basal plates of mature onion bulbs (Fig. 1A).

Conidial inoculation of transversely cut basal plates was compared with that of basal plates that had only the dead roots removed; the rest of the basal plate remained intact (Fig. 1B). In both cases, inoculation was performed using three different conidial concentrations (3×10^5, 3×10^4, and 3×10^3 spores/mL). The incubation environment and scoring were the same as described for conidial inoculation. This experiment was performed out in a factorial design with three replications.

Selection and evaluation of FBR resistance. A schematic diagram of the phenotypic recurrent selection and evaluation of FBR resistance is provided in Fig. 2. Harvested bulbs were stored for 4 weeks to discard any naturally infected bulbs (Mandal et al., 2020). Then, the remaining dormant bulbs were artificially inoculated using the mycelial inoculation method described previously, and the inoculated bulbs were placed in a greenhouse (temperature, 25 to 30 °C; relative humidity, 70%) (Saxena, 2009). After incubating for 20 d, disease-free bulbs were selected. These inoculation and selection methods were conducted again for the remaining bulbs. After two rounds of

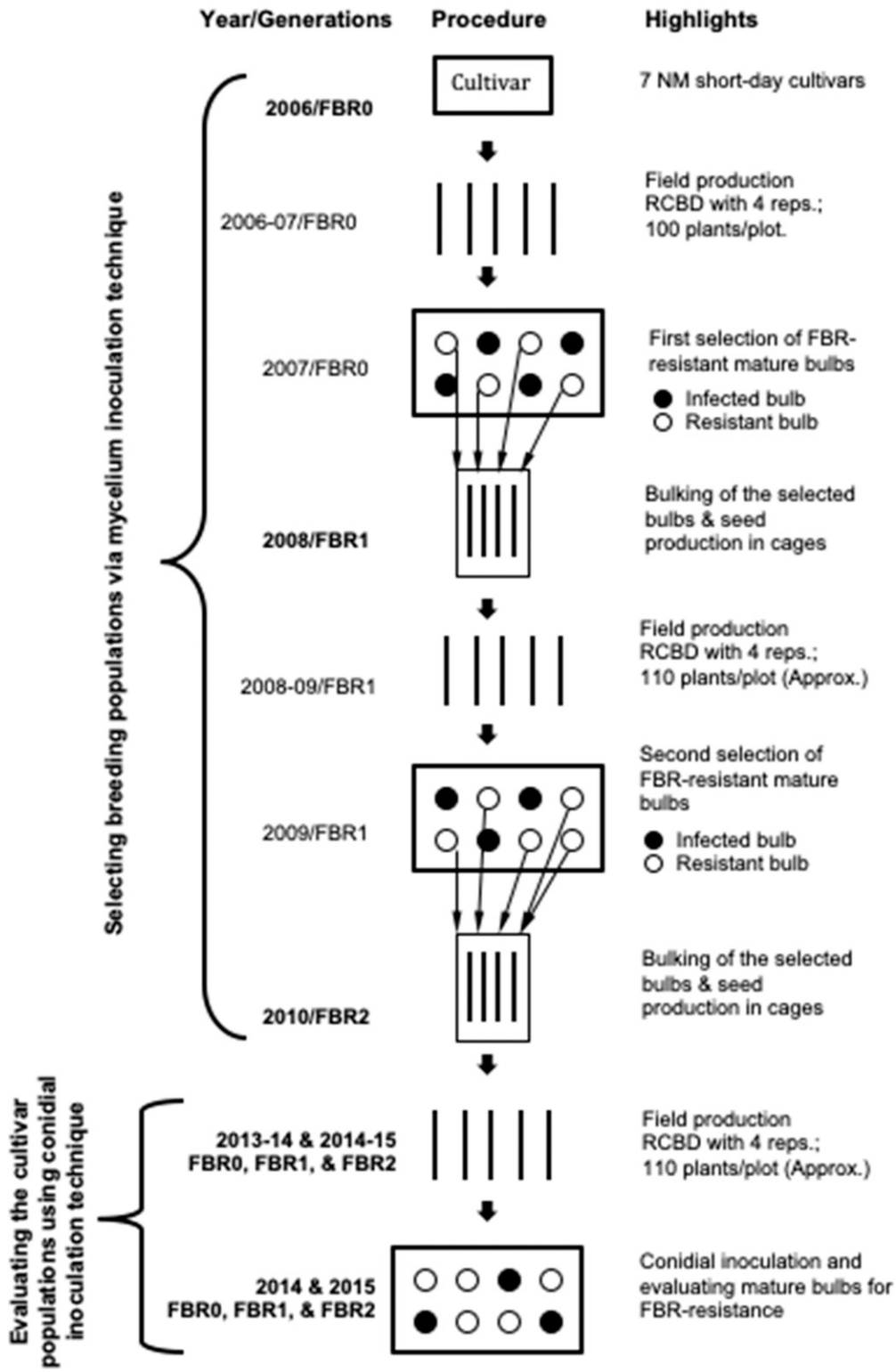

Fig. 2. Flow chart of the selection cycle and evaluation of a cultivar population via artificial inoculation of mature onion bulbs.

selection, disease-free bulbs were bulked and planted inside insect-proof cages to obtain seeds (Mandal et al., 2020). The production of seeds concluded one cycle of selection in 2008 to produce the FBR1 generation from the FBR0 generation. This entire selection process was repeated to produce the FBR2 generation from the FBR1 generation in 2010.

An evaluation of FBR resistance was conducted in a large wooden onion shed during Summer 2014 and 2015. Dormant bulbs were artificially inoculated using the conidial inoculation method and placed with the cut side up in black plastic crates ($58 \times 37 \times 22$ cm^3) (Bekuplast GmbH, Ringe, Germany). Crates were placed inside black polyethylene bags ($83.8 \times 104 \times 0.00254$ cm^3; Poly-America Grand Prairie, TX) for 72 h (in 2014) or 24 h (in 2015) to increase humidity (≈85%), and bulbs were incubated for 20 d. The high summer temperatures in New Mexico and well-ventilated shelves inside the shed allowed for proper disease development. Re-inoculation was performed if the percent incidence was not more than 90% for the susceptible control to avoid false-positive

results. On day 21, FBR infection on the basal plate (i.e., severity) was visually rated using a scale of 1 (no disease) to 9 (≥70% diseased basal plate tissue by area) (Table 2).

Statistical analysis. Statistical analysis was performed using SAS Studio in a Web-based environment called SAS OnDemand for Academics (SAS Institute Inc., Cary, NC). Plot means of disease incidence (%) were calculated as the percentage of inoculated bulbs exhibiting FBR symptoms. Mean plot FBR severity and incidence were calculated for 20 arbitrarily selected bulbs. Then, these plot means were used to calculate variance among seasons, cultivar populations, and their interactions using the Proc GLM statement. Specific pairwise differences were calculated for cultivar populations and controls using the Proc GLM contrast statement. Proc GLM was used for the experiment comparing transversely cut and undisturbed basal plate inoculations to obtain the main effects of the basal plate and conidial concentrations on disease development, followed by Fisher's least significant difference for mean separation of disease severity and incidence at different levels of spore concentrations and basal plate combinations.

Results

FBR susceptibility of the cultivars revealed by conidial inoculation. FBR severity and incidence varied among the cultivar populations over the years and were influenced by their interactions (Table 3). The susceptible and tolerant controls exhibited a similar FBR severity or incidence in 2014, and they only differed in 2015 due to reductions in the spore concentration and period of high-humidity incubation after inoculation in the later year (Fig. 3). Nevertheless, the control and seven cultivar populations exhibited a high degree of disease severity and incidence in both seasons, showing the efficiency of the conidial inoculation in disease development (Fig. 3). 'NuMex Mesa' (severity, 4.5–5.5) populations exhibited lower FBR severity than both the susceptible and tolerant controls (severity, 7.9) in 2014. However, 'NuMex Camino' (severity, 7.3–8.4; incidence, 85.7% to 96.3%) and 'NuMex Luna' (severity, 7.6–8.7; incidence, 86.3% to 98.4%) exhibited comparable or higher FBR severity and incidence than the susceptible control (severity, 7.4–8.1; incidence, 88.8% to 92.5%) in both seasons, indicating that both are very susceptible cultivars (Fig. 3).

Conidial inoculation evaluation underscored the constraints of the mycelium inoculation to increase FBR resistance. The conidial inoculation evaluation revealed a few instances when lower disease severity and/or incidence were observed in the selected populations when compared with the original populations. In 2014, the severity and incidence were lower for the FBR1 populations compared with the FBR0 populations of 'NuMex Sweetpak' and 'NuMex Chaco' (Fig. 3). Reductions were not observed for disease severity or incidence for the FBR2 populations of both cultivars in 2015 (Fig. 3). In 2014, higher severity and incidence were observed for the FBR1 population of 'NuMex Crispy' than for the FBR0 population (Fig. 3); these were not observed in 2015 due to the unavailability of FBR0 bulbs (Fig. 3).

Optimum inoculum density for conidial inoculation and mechanical resistance of undisturbed basal plate tissue. The basal plate (removing the basal plate top vs. removing only dead roots), spore concentration, and their interactions were the determinants of successful disease development after conidial inoculation (Table 4). Lower FBR severity (by 41.8%) and incidence (by 50%) were observed when the FOC spore concentration was reduced from 3×10^5 to 3×10^4 spores/mL in the conidial inoculation with the removal of basal plate top (Figs. 1A and 4). No change in the FBR disease parameters was observed after another 10-fold reduction in the spore concentration from 3×10^4 to 3×10^3 (Fig. 4). A minimal amount of FBR in the intact basal plates without dead roots suggested that conidial inoculation of intact basal plates is not an effective method of screening for resistant bulbs (Fig. 4).

Table 2. Visual rating scale for calculating the severity of fusarium basal rot (FBR).

Levels[z]	Percentage of basal plate infected with FBR
1	No infection
2	1% to 10%
3	11% to 20%
4	21% to 30%
5	31% to 40%
6	41% to 50%
7	51% to 60%
8	61% to 70%
9	>70%

[z]Symptoms of each level on the basal plate can be found in Gutierrez and Cramer (2005).

Table 3. Analysis of variance (mean squares) for fusarium basal rot (FBR) severity and incidence after conidial inoculation of seven onion cultivars and their selections at Las Cruces, NM, in 2014 and 2015.

Source[z]	Degrees of freedom	Mean squares	
		Disease severity[y]	Disease incidence[x]
Year	1	14.9***	1220.1**
Lines	20	3.9***	463.2***
Year × line	19	2.9***	347.1***
Block	3	0.8[NS]	70.8[NS]
Year × block	3	3.4*	417.9*
Error	112	0.9	

[z]Seven cultivars and their two selections each were evaluated for their FBR severity and incidence in a randomized complete block design with four replications for two seasons.
[y]FBR severity was calculated after 20 d of incubation. Twenty arbitrarily selected *Fusarium oxysporum* f.sp. *cepae* (Hanzawa) Snyder & Hansen (FOC)-inoculated bulbs were rated individually for FBR severity on a scale of 1 to 9 (1 = no symptoms; 9 = ≥70% of the basal plate is infected) (Gutierrez and Cramer, 2005).
[x]FBR incidence, which is the percentage of 20 inoculated bulbs showing FBR symptoms, was calculated after 20 d of incubation.
[NS, *, **, ***]Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

Discussion

The conidial inoculation method was very effective for producing reproducible FBR symptoms in mature bulbs of all the cultivars, including the controls. This is an essential consideration for the artificial screening of resistant germplasm by eliminating disease escape and imitating natural infestation (Schroeder et al., 2010). In addition to ample infection, genetic variations of FBR resistance by the cultivars revealed that inoculating basal plates with FOC conidia embedded in PDA was as effective as injecting fleshy scales with *Enterobacter cloacae* inoculum for storage rot (Schroeder et al., 2010). In both cases, the inoculation methods did not suppress the inherent biological mechanisms of resistance and allowed the expression of different genetic responses by the cultivars. Therefore, the conidial inoculation developed in this study could replace an unreliable field inoculation for screening mature bulbs for resistance to FBR. In the past field trials at the FGSC, lower FBR severity and incidence were demonstrated by the same seven entries (Cramer and Muhyi, 2002; Cramer et al., 2000, 2001, 2002; Gutierrez and Cramer, 2005) due to the nonuniform distribution of FOC in the fields (Saxena, 2007). Reducing the conidial concentration further from 3×10^5 spores/mL to 3×10^4 spores/mL could detect subtle differences between the cultivars because fewer propagules were observed in natural FBR infection (Abawi and Lorbeer, 1971a).

Mycelial inoculation failed to improve FBR resistance for the FBR1 and FBR2 selected cultivar populations, which suggested the possibility of disease escape by the susceptible bulbs. Maintaining uniform propagule pressure was not possible with this inoculation method due to the variable growth of fungal mycelium and sporulation on nutrient-rich PDA media (Sharma and Pandey, 2010; Su et al., 2012). Variable inoculum density could result in differential disease development, as evidenced by a range of black sigatoka disease severity on banana leaves, depending on mycelial densities and fragmentation patterns of a single isolate of *Mycosphaerella fijiensis* (Donzelli and Churchill, 2007). Therefore, disease escape by a single susceptible bulb after mycelial

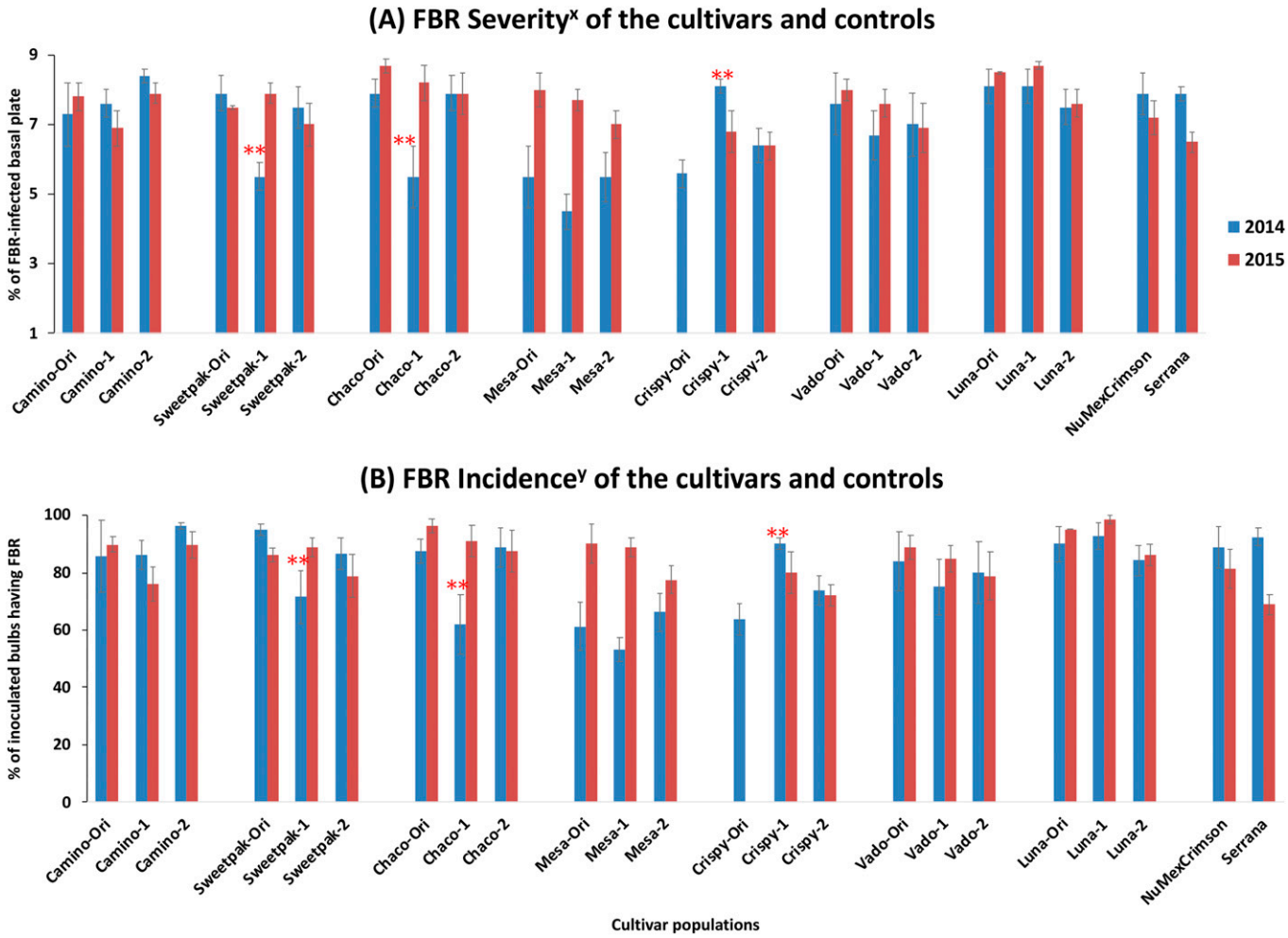

Fig. 3. Fusarium basal rot (FBR) severity and incidence of mature bulbs of New Mexico short-day early-maturing cultivars along with two controls: (**A** and **B**) NuMex Camino and (**C** and **D**) NuMex Sweetpak. [x]FBR severity was calculated after 20 d of incubation. Twenty randomly selected FOC-inoculated bulbs were rated individually for FBR severity on a scale of 1 to 9 (1 = no symptoms; 9 = ≥70% of the basal plate is infected) (Gutierrez and Cramer, 2005). [y]FBR incidence, which is the percentage of 20 inoculated bulbs showing FBR symptoms, was calculated after 20 d of incubation. [Ori, 1, 2]Original, first selected generation, and second selected generation, respectively. [Crispy-Ori]An evaluation of the original populations of NuMex Crispy was not possible in 2015 due to the lack of sufficient bulb production. **Significantly different from FBR0 at $P \leq 0.01$.

Table 4. Analysis of variance (mean squares) of disease severity and incidence (%) of the two basal plate types (cut vs. intact) and three *Fusarium oxysporum* f.sp. *cepae* (Hanzawa) Snyder & Hansen (FOC) concentrations and their interactions.

		Mean squares	
Source[z]	Degrees of freedom	Disease severity[y]	Disease incidence[x]
Basal plates	1	19.6***	2037.3***
Spore concentrations	2	6.7***	1237.8***
Basal plate × spore concentrations	2	5.1***	650.0**
Replications	2	0.02[NS]	24.5[NS]
Error	10	0.38	94.3

[z]Basal plates types and FOC spore concentrations are the two main effects. There are two levels of basal plates (cut and intact) and three levels of spore concentrations (3×10^5, 3×10^4, and 3×10^3 spores/mL) included.
[y]FBR severity was calculated after 20 d of incubation. Twenty arbitrarily selected FOC-inoculated bulbs were rated individually for FBR severity on a scale of 1 to 9 (1 = no symptoms; 9 = ≥70% of the basal plate is infected (Gutierrez and Cramer, 2005).
[x]FBR incidence, which is the percentage of 20 inoculated bulbs showing FBR symptoms, was calculated after 20 d of incubation.
[NS, **, ***]Nonsignificant or significant at $P < 0.01$ or 0.001, respectively.

inoculation could jeopardize any improvement by selection due to the outcrossing nature of this crop. In contrast, every bulb was challenged by the same amount of FOC spores in the conidial inoculation to eliminate the possibility of disease escape. Due to the difficulties related to mycelial quantification and inoculum delivery, conidial inoculation is preferred in many fungal disease resistance studies of plants, such as black sigatoka disease in banana (Twizeyimana et al., 2007), boxwood blight (Guo et al., 2016), and premature ripening of sunflower (Seassau et al., 2010).

Initial fungal establishment for causing infection would be difficult for mycelial inoculation because the inoculated bulbs were kept open in a greenhouse (Saxena, 2009). This exposure of the PDA plugs to direct sunlight could dry the inoculation surface prematurely within 5 h before successful pathogen invasion (Shokes et al., 1996; Xu and Ko, 1998). Therefore, desiccation of the inoculated surface and PDA plugs was delayed up to 72 h by the conidial inoculation using polyethylene bags. Seventy-two hours of high humidity around the artificially inoculated surface resulted in successful development of sheath blight infection in rice by *Rhizoctonia solani* (Park et al., 2008) and leaf and stem rot in pepper by *Phytopthora capsici* (Xu and Ko, 1998). The delayed desiccation of PDA and the cut basal plate with the conidial inoculation provided sufficient time for the embedded fungal spores to

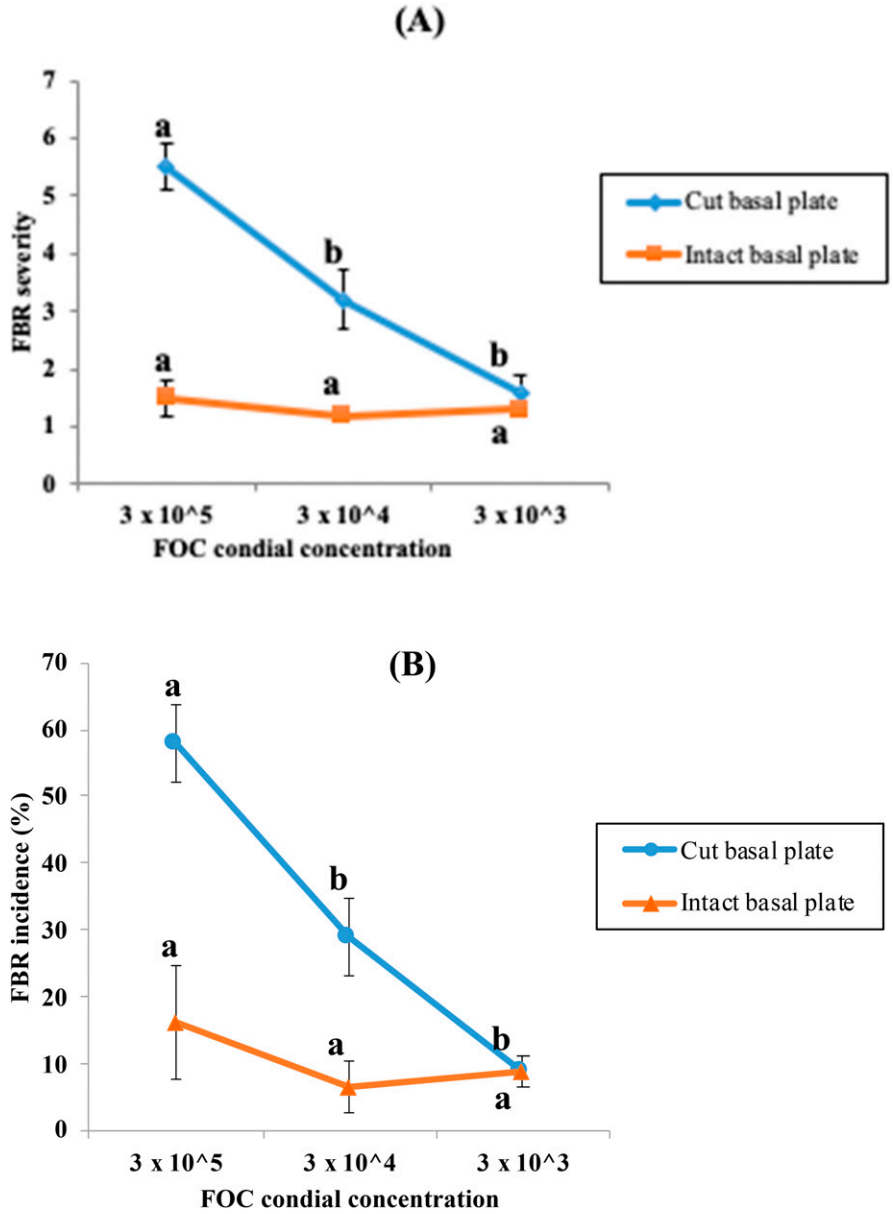

Fig. 4. Changes in (**A**) fusarium basal rot (FBR) severity and (**B**) FBR incidence depending on basal plate conditions and conidial concentrations. *Fusarium oxysporum* f.sp. *cepae* (Hanzawa) Snyder & Hansen (FOC) conidia were suspended in potato dextrose agar media in three different concentrations (3×10^5, 3×10^4, and 3×10^3 spores/mL). The cut basal plate method involves transversely cutting a thin section (≈0.25–0.30 mm) of the tissue from the upper surface; the intact basal plate method includes the intact tissue. With each basal plate condition, the same letter indicates a nonsignificant difference in FBR severity and incidence at $P < 0.05$ of two conidial concentrations. Vertical bars indicate ± SE (n = 3; r = average of 15 bulbs).

germinate and invade the inoculated surface before forming a physical barrier to prevent infection (Fig. 4). It helped produce higher microconidia, macroconidia, and cfu in ≈85% relative humidity (Gracia-Garza and Fravel, 1998) and subsequently caused ample FBR infection.

Very high fungal virulence was maintained for conidial inoculation. To avoid declining levels of virulence due to successive subcultures on artificial media (Hajek et al., 1990) and changing morphology of fungus due to long-term storage (Hajek et al., 1995), the high virulence of the FOC isolate was assured periodically by restricting subculture to only one time and inoculating sterile bulbs, respectively. Because deterioration of the basal plate by FOC is an enzymatic activity (Holz and Knox-Davies, 1985a) that secures apoplast sugar (Holz and Knox-Davies, 1986), the molecular or biochemical mechanisms responsible for producing those enzymes could be reactivated effectively using basal plate tissue before culturing the fungus onto PDA media. Therefore, the FOC subculture produced from an infected basal plate tissue would be more virulent than if produced from PDA media. The loss of virulence, which results in no disease development by a pathogenic isolate, can be measured using a susceptible control. Unlike conidial inoculation, a decrease in the virulence of the FOC isolate with multiple subculturing cycles could not be substantiated in the mycelial inoculation due to the absence of any susceptible control (Saxena, 2009).

The preliminary data regarding conidial inoculation of the undisturbed basal plate method showed insufficient FBR infection. Other attempts to inoculate intact basal plates with FOC-infected oat seeds coated with Arabic gum or molasses and polyacrylamide gel containing a spore suspension also resulted in insignificant or no disease development (data not presented). Failure to infect intact basal plates in storage could imply mechanical resistance (Agrios, 2005) conferred by the rough and sturdy outer layer, which could only be overcome by complete basal plate top removal (Holz and Knox-Davies, 1969) or basal plate puncture (Somkuwar et al., 1996). In natural soil, FOC can infect both resistant and susceptible cultivars equally by invading either its roots or naturally wounded basal plate caused by continuous death of roots (Abawi and Lorbeer, 1971b). FBR-infected bulbs with intact root systems were also seen during FGSC field trials, which indicated that FOC could enter the soft tissue of the basal plate via cracks developed due to bulb growth expansion or abiotic stress (C.S. Cramer, personal communication). These wounds could be healed by suberin-containing structural barriers, such as periderm, and protect the plant from pathogenic infections (Soler et al., 2011). Because wound-induced suberization accelerates in the presence of higher oxygen (Wei et al., 2018), FOC penetration is more difficult in storage than in soil if the suberization rate of the external basal plate tissue outcompetes the rate of pathogenic development. A histological study of basal plate penetration after artificial inoculation of mature bulbs during storage could indicate the reason for the mechanical resistance of FOC.

Conclusion and future breeding. This study developed a conidial inoculation method to screen FBR-resistant mature onion bulbs. This inoculation method produced an ample amount of reproducible FBR infection, mimicked natural symptoms, and did not circumvent natural infection in genetically different short-day onion cultivars. Additionally, this method eliminated the possibility of any disease escape and could replace field screening for a valid selection of FBR-resistant onion bulbs.

Because high disease pressure eventually increases disease resistance in progeny with intense selection pressure (Pratt, 1996), surviving mature bulbs of each entry are more resistant than their respective parents. Furthermore, increasing the selection gain by identifying the most resistant materials in a population (Pratt, 1996) would likely reduce the number of cycles required to achieve a high degree of FBR resistance by the short-day onion cultivars.

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