Screening Winter-sown, Intermediate-day Onion Cultivars for Resistance to Fusarium Basal Rot

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SUMMARY. Fusarium basal rot (FBR), caused by *Fusarium oxysporum* f.sp. *cepae*, is a soilborne fungal disease that affects bulb onions (*Allium cepa*) worldwide. Winter-sown onion cultivars that are resistant to FBR are lacking. The goal of this project was to screen winter-sown onion germplasm for FBR resistance using a mature-bulb field screening at harvest and after 4 weeks in storage. The project was conducted for 2 years, and in each year, 22 winter-sown onion lines were grown in a field known to produce a high incidence of FBR-infected bulbs. At maturity, the basal plates of 20 randomly selected bulbs were cut transversely and each plate was scored for disease severity on a scale of 1 (no diseased tissue) to 9 (70% or more diseased tissue). Bulbs were stored and scored again at 4 weeks after harvest. Severity and incidence increased in storage for both years. NMSU 99-30, 'NuMex Arthur', and 'NuMex Jose Fernandez' showed the lowest disease severities and incidences in both years. For fields that produce a high incidence of FBR-infected bulbs, these cultivars could be grown with less loss to FBR at harvest and after storage than more FBR-susceptible cultivars. When developing FBR-resistant cultivars, breeding lines should be evaluated over multiple years and bulbs should be stored for 4 weeks before being screened.

Fusarium basal rot, caused by *Fusarium oxysporum* f. sp. *cepae* (FOC), is a soilborne disease affecting onions worldwide, causing significant economic losses in Italy, Japan, South Africa, and the United States (Sumner, 1995). Onions grown on soils naturally infested with FOC can have a 23% field and storage loss (Bacher et al., 1989). In New Mexico, FBR is the second most important soilborne disease affecting onions (N. Goldberg, personal communication). For FBR-susceptible cultivars, disease incidence can be substantial when they are grown in highly infested fields. 'Cardinal', a FBR-susceptible cultivar, exhibited 64% to 75% disease incidence in 2 years when grown in highly infested FBR fields in New Mexico (Cramer and

Muyhi, 2002; Cramer et al., 2002).

The fungus attacks seedlings, causing pre- and postemergence damping-off. In addition, root rot of older plants, stem plate discoloration, and basal rot of bulbs can be observed in the field and in storage (Abawi and Lorbeer, 1972). Disease development is optimized when soil temperature ranges from 25 to 28 °C (Sumner, 1995). In Las Cruces, N.M., soil temperatures are usually at these levels from April until October. Infection by FOC can occur through wounding or by direct penetration by the fungus through the basal stem plate.

Losses from infection by FOC can be reduced by rotation with non-host crops in infested fields (Sumner, 1995) or by soil fumigation, which has been effective but not economical (Bacher et al., 1989). In New Mexico, crop rotation has been an effective means

of controlling FBR such that incidence levels are 5% or less. However, ideal cropland is being lost to perennial crops such as pecans (*Carya illinoensis*) and alfalfa (*Medicago sativa*) and also is being lost to urban development. With this loss of cropland, crop rotation cycles are shortening and fields are having repeated onion culture in a shorter period of time than previously. With repeated onion culture over a short period of time, FBR disease incidence will increase in New Mexico onion crops. Once crop rotation is compromised as a method of disease control, the next control measure is the use of resistant onion cultivars (Sumner, 1995). However, winter-sown, intermediate-day onion cultivars have not been screened for FBR resistance when they are grown in highly infested FBR soils.

The goal of this project was to screen winter-sown, intermediate-day onion germplasm for FBR resistance using mature-bulb field screening and to find cultivars that possessed resistance. Mature bulbs were chosen for rating because they are the most FBR-susceptible stage of onion plant development (Holz and Knox-Davies, 1974). Mature bulbs were to be screened at harvest and after 4 weeks of storage. We wanted to observe disease progression over time. An after-harvest screening date was chosen because in our preliminary studies, disease incidence was observed to increase after bulbs were stored for a period of time and because other onion researchers have delayed screening up to 5 months after harvest (Abawi and Lorbeer, 1971; Holz and Knox-Davies, 1974; Lorbeer and Stone, 1965; Marlatt, 1958; Retig et al., 1970; Stadnik and Dhingra, 1996).

Materials and methods

Twenty-two winter-sown, intermediate-day onion cultivars were grown in fields known to produce a high incidence of FBR-infected bulbs at the Fabian Garcia Research Center in Las Cruces, N.M. These cultivars

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.5400	inch(es)	cm	0.3937
1.1209	lb/acre	kg·ha ⁻¹	0.8922
1	ppm	mg·kg ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

were chosen because they were cultivars being used in commercial production in New Mexico or they were breeding lines being developed into cultivars by the New Mexico State University (NMSU) onion breeding program. In a 3-year period, FBR-susceptible cultivars expressed a disease incidence at harvest of 64% to 99% when grown on these fields (Cramer and Muhyi, 2002; Cramer et al., 2001, 2002). The high inoculum level is due to infected bulbs being incorporated into field soil after each growing season; onions being successively sown in the same fields; and fungicides not being applied to control the pathogen and its disease. Prior to this study, *FOC* was isolated from symptomatic basal plate tissue and confirmed as the causal agent of the diseased tissue (Lopez, 2003). Entries were seeded on 16 Jan. 2001 and 7 Jan. 2002, and thinned on 25 Feb. 2001 and 16 Feb. 2002, respectively, to 4-inch in-row spacing. Each entry was seeded into beds formed 1 m center-to-center. Each plot was 8 ft long with two rows spaced 8 inches apart. Entries were arranged in a randomized complete-block design with four replications per entry. Fields were managed using standard cultural practices for producing onions in southern New Mexico (Corgan et al., 2000). Preplant, 250 lb/acre of triple superphosphate (0N-20.1P-0K) was applied. Subsurface drip irrigation tape, placed 4 inches below the surface with 8 inches between emitters (T-Tape; T-Systems International, San Diego), was used to apply water as needed. Uran 32 (urea and ammonium nitrate 32N-0P-0K; Helena Chemical Co., Collierville, Tenn.) was applied at 20–30 mg·kg⁻¹ through drip lines for a total of 200–250 lb/acre N for the crop. This fertilization rate is common for onion production on the sandy loam, mineral soils of southern New Mexico (Corgan et al., 2000).

Bulb maturity was determined when approximately 80% of the tops within a plot had fallen. At maturity, bulbs were harvested, and the plant tops and roots were clipped from the bulb. The basal plates of 20 randomly selected bulbs from the plot were cut transversely through the basal plate and rated for disease severity on a scale of 1 (no diseased tissue) to 9 (70% or more diseased tissue) (Gutierrez and Cramer, 2005). Only in the 2002 season, knives were surface sterilized in an aqueous

solution of 20% bleach (6% sodium hypochlorite), and dried between each cut, to avoid the spread of *FOC* from infected bulbs to non-infected bulbs. After basal plates were rated, bulbs from the plot were placed into individual paper bags. Data from the 20 bulbs from each plot were used to calculate the mean disease severity for each entry. The average FBR incidence per plot was calculated from the percentage of sampled bulbs that showed any sign of infection by *FOC*.

After basal plates were rated, bulbs were placed into individual paper bags and stored at ambient conditions (25 to 32 °C) for 4 weeks. Bulbs that were rated with a score of 9 at harvest were included for calculating severity and incidence at harvest but not stored for the disease progression study. Bulbs discarded for this reason were replaced with a new bulb that was rated, and stored. After 4 weeks of storage, the entire basal plate of bulbs was recut and basal plates were rated for FBR severity. The same 20 bulbs were rated at 4 weeks so that disease progression could be observed for each bulb. The length of 4 weeks in storage prior to rating was chosen for several reasons. Based on previous observations, 4 weeks was enough time to allow disease development. Storage beyond 4 weeks would allow for the development of bacterial diseases that would make the FBR disease rating more difficult. A mean incidence for each cultivar was calculated from the average FBR incidence per plot. The means for each trait were calculated using the means procedure of SAS statistical software (SAS Institute, Cary, N.C.). Within each group, differences between entries were calculated for each trait using the SAS general linear model procedure. Mean separation was determined with Fisher's least significant difference (LSD) test ($P \leq 0.05$).

Results and discussion

For each of the traits measured, entries exhibited different responses depending upon the year tested. A significant year by cultivar interaction was observed for three of the four traits. For this reason, results for each year will be discussed separately by cultivar for disease severity and incidence at harvest and disease severity after 4 weeks of storage rather than averaged over years. Disease incidence was measured at 4 weeks after storage but was not

significantly different between years; therefore, incidence at 4 weeks was averaged over both years. All entries exhibited an increased incidence after 4 weeks of storage for both years. The main difference between years resulted from higher severity ratings and incidences in 2001 than in 2002. Nevertheless, there were instances where particular entries changed rank from one year to the next. In general, entries that had the lowest severities also had the lowest incidences at harvest.

2001 SEASON. The mean severity at harvest was 1.5 and the mean incidence was 17.2% for all entries (Table 1). The majority of entries exhibited low FBR severity and incidence of infection at harvest. NMSU 99-32, 'NuMex Arthur', and 'Utopia' exhibited lower disease severity than 'Alabaster', 'Aspen', 'Frosty', NMSU 99-33-1, 'NuMex Casper', and 'Tara' (Table 1). In a previous NMSU winter-sown cultivar trial, 'Utopia' had exhibited a low FBR severity at harvest (Cramer et al., 2002). In addition, 'NuMex Jose Fernandez' and 'NuMex Snowball' exhibited a lower disease severity than 'Alabaster', 'Aspen', 'Frosty', 'NuMex Casper', and 'Tara'. 'Aspen' exhibited a higher FBR severity at harvest than all other entries except 'Alabaster' and 'Tara'. NMSU 99-32, 'NuMex Arthur', and 'Utopia' exhibited a lower disease incidence at harvest than eight other entries (Table 1). In addition, 'NuMex Jose Fernandez' exhibited a lower incidence than 'Alabaster', 'Aspen', 'Frosty', NMSU 99-33-1, and 'NuMex Casper'; while 'NuMex Snowball' exhibited a lower incidence than 'Alabaster', 'Aspen', NMSU 99-33-1, and 'NuMex Casper'. Of the cultivars tested, NMSU 99-32, 'NuMex Arthur', 'NuMex Jose Fernandez', 'NuMex Snowball', and 'Utopia' exhibited the lowest FBR severity and incidence at harvest and show the greatest potential for FBR resistance.

After 4 weeks of storage, FBR severity levels increased for all 22 entries (Table 1). The average disease severity was 4.9 at 4 weeks compared to 1.5 at harvest. Other researchers also have observed an increase in FBR symptoms with storage (Gutierrez and Cramer, 2005; Marlatt, 1958; Retig et al., 1970; Stadnik and Dhingra, 1996). NMSU 99-30 exhibited a lower disease severity after 4 weeks of storage than all other cultivars. In addition, 'NuMex

Table 1. *Fusarium* basal rot severity rating at harvest, rating after 4 weeks of storage, and incidence at harvest for 22 winter-sown, intermediate-day onion entries during 2001 at Las Cruces, N.M.

Entry	Severity at harvest ^z	Severity at 4 weeks ^y	Incidence at harvest (%) ^x
Alabaster	2.0	6.7	31.3
Aspen	2.5	7.8	33.8
Caballero	1.3	4.3	12.5
Candy	1.4	4.2	17.5
Cimarron	1.3	5.1	15.0
Dawn	1.5	5.4	11.3
Frosty	1.9	6.0	22.5
Mesquite	1.5	4.1	16.3
NMSU 00-28-1	1.4	4.1	13.8
NMSU 99-30	1.3	2.1	10.0
NMSU 99-32	1.1	5.2	5.0
NMSU 99-33-1	1.7	5.2	27.5
NuMex Arthur	1.1	3.4	5.0
NuMex Bolo	1.3	3.8	16.3
NuMex Casper	1.8	3.7	26.3
NuMex Jose Fernandez	1.2	3.3	7.5
NuMex Snowball	1.2	4.3	8.8
Riviera	1.4	5.9	16.3
Sierra Blanca	1.3	6.8	20.0
Tara	2.1	5.3	21.3
Tequila	1.6	4.5	20.0
Utopia	1.1	4.4	5.0
Mean	1.5	4.9	17.2
LSD (5%)	0.6***	1.2***	14.9***

^xCut basal plates of 20 randomly selected bulbs per plot (four replicates each) were rated based on a scale of 1 (no diseased tissue) to 9 (70% or more of basal plate decayed).

^yBasal plates of same 20 bulbs were recut and rerated after 4 weeks in storage.

^zPercentage of bulbs with *Fusarium* basal plate rot.

***Significant at $P = 0.001$.

Arthur' and 'NuMex Jose Fernandez' exhibited a lower disease severity after 4 weeks of storage than 10 other entries. These three entries also exhibited a low disease severity and incidence at harvest. 'Aspen' exhibited a disease severity after 4 weeks of 7.8 that was greater than all other entries except 'Alabaster' and 'Sierra Blanca' (Table 1). In addition, 'Alabaster' and 'Sierra Blanca' exhibited a disease severity after 4 weeks of storage that was greater than the severity of all other entries except 'Frosty' and 'Riviera' (Table 1). 'Aspen' and 'Alabaster' also had exhibited a disease severity and incidence at harvest that was greater than the severity and incidence of other entries. However, 'Sierra Blanca' had exhibited a low disease severity at harvest but severity increased substantially after 4 weeks of storage. NMSU 99-32 and 'Riviera' followed the trend of 'Sierra Blanca' by exhibiting a low disease severity at harvest but moderate disease severity after 4 weeks of storage. From

these results, FBR severity increases with storage; however, the degree of increase is cultivar dependent. Selection for FBR-resistant bulbs should be conducted after bulbs have been stored for at least 4 weeks. Under our growing conditions in New Mexico, NMSU 99-30, 'NuMex Arthur', and 'NuMex Jose Fernandez' appear to be more resistant to FBR than other intermediate-day cultivars.

2002 SEASON. In the second season, the mean severity and incidence decreased as compared to the previous year with values of 1.3% and 9.5%, respectively (Table 2). Ten entries exhibited a disease incidence at harvest of 5% or less (Table 2). 'Frosty' exhibited a disease severity and incidence at harvest that was greater than the severity and incidence of all other cultivars. In addition, 'Mesquite', 'NuMex Casper', and 'Tequila' exhibited a disease severity that was greater than the severity of 14 other entries, although the difference was small. In addition, 'Mesquite'

Table 2. *Fusarium* basal rot severity rating at harvest, and rating after 4 weeks of storage, and incidence at harvest for 22 winter-sown, intermediate-day, onion entries during 2002 at Las Cruces, N.M.

Entry	Severity at harvest ^z	Severity at 4 weeks ^y	Incidence at harvest (%) ^x
Alabaster	1.2	4.6	7.5
Aspen	1.2	4.5	5.0
Caballero	1.2	4.3	6.3
Candy	1.1	2.6	3.8
Cimarron	1.2	4.3	7.5
Dawn	1.0	3.4	0.0
Frosty	2.4	4.7	38.8
Mesquite	1.6	5.1	21.3
NMSU 00-28-1	1.1	4.5	5.0
NMSU 99-30	1.3	2.2	11.3
NMSU 99-32	1.0	3.4	1.3
NMSU 99-33-1	1.4	3.0	8.8
NuMex Arthur	1.2	2.7	5.0
NuMex Bolo	1.2	3.0	5.0
NuMex Casper	1.6	3.2	16.3
NuMex Jose Fernandez	1.0	2.0	1.3
NuMex Snowball	1.6	3.8	18.8
Riviera	1.0	5.1	2.5
Sierra Blanca	1.3	4.1	11.3
Tara	1.2	4.5	10.0
Tequila	1.6	3.9	16.3
Utopia	1.2	3.4	5.0
Mean	1.3	3.7	9.5
LSD (5%)	0.4***	1.5***	9.0***

^xCut basal plates of 20 randomly selected bulbs per plot (four replicates each) were rated based on a scale of 1 (no diseased tissue) to 9 (70% or more of basal plate decayed).

^yBasal plates of same 20 bulbs were recut and rerated after 4 weeks in storage.

^zPercentage of bulbs with *Fusarium* basal plate rot.

***Significant at $P = 0.001$.

exhibited a disease incidence at harvest that was greater than the incidence of all other entries except 'NuMex Casper', 'NuMex Snowball', and 'Tequila'.

As observed in the first year, the average disease severity of all entries in the second year increased from 1.3 to 3.7 after 4 weeks of storage (Table 2). NMSU 99-30 and 'NuMex Jose Fernandez' exhibited a disease severity after 4 weeks of storage that was less than the severity of all but eight other entries. 'Mesquite' exhibited a disease severity after 4 weeks of storage that was greater than the severity of the aforementioned cultivars. As observed in the first year, a number of entries, such as 'Alabaster', 'Aspen', 'Riviera', and 'Tara', that had exhibited a low disease severity at harvest, exhibited moderate disease severities after 4 weeks of storage. As was observed in the previous year, NMSU 99-30 and 'NuMex Jose Fernandez' exhibited little change in disease severity after bulbs were stored for 4 weeks.

The use of bleach to surface sterilize cutting knives in-between bulb samples in the 2002 season but not in 2001 season did not seem to affect the outcome of the results. Differential treatment of the cutting instrument between years should not influence disease ratings at harvest since disease symptoms at harvest are present prior to cutting the basal plate and at least 1 week would be required after cutting for disease development resulting from the transfer of spores from bulb to bulb through the use of an infected knife. However, yearly differences were observed at harvest for disease severity and incidence and the treatment of the cutting instrument would not have caused these yearly differences. Differential treatment of the cutting instrument between years might influence disease incidence after bulbs have been stored for 4 weeks. However, there was no difference between years in the disease incidence after 4 weeks of bulb storage. In addition, even if there had been differences between years in disease incidence that were correlated with bleach treatment of the knives, the determination of FBR-resistant entries should not be affected. Truly resistant bulbs should not express disease symptoms when challenged with the pathogen.

DISEASE INCIDENCE AFTER 4 WEEKS OF STORAGE. As mentioned earlier, there was no significant cultivar by year interaction for disease incidence after 4 weeks of storage, so disease incidence of entries was averaged over years (Table 3). As with disease severity, disease incidence increased after 4 weeks of storage as compared to the incidence at harvest in either year. NMSU 99-30 exhibited a lower disease incidence after 4 weeks of storage than the incidence of all other cultivars except 'NuMex Jose Fernandez' (Table 3). In addition, 'NuMex Jose Fernandez' exhibited a lower disease incidence after 4 weeks of storage than the incidence of all but seven other entries. The disease incidence of 'Riviera' was greater than the incidence of all but eight other entries (Table 3). An increase in disease incidence after 4 weeks of storage indicates that more bulbs are expressing disease symptoms after storage than at harvest time with the disease-causing organism present at harvest, but is not causing visible disease symptoms. As indicated earlier, selection at harvest time for FBR-resistant bulbs is problematic if

symptoms are not being expressed. Bulbs of NMSU 99-30 and 'NuMex Jose Fernandez' appear to be more resistant to FBR than other entries tested. These two entries would be logical choices for the development of FBR-resistant, intermediate-day cultivars.

The three entries, NMSU 99-30, 'NuMex Arthur', and 'NuMex Jose Fernandez', that expressed the highest levels of FBR resistance, have several characteristics in common that might partially explain their higher levels of resistance than the other entries. These three entries were developed by the NMSU onion breeding program. In the selection and development phase, these entries were grown in fields known to be infested with high levels of the pathogen that causes FBR. The repeat selection of bulbs grown in these fields has resulted a higher level of FBR resistance than is found in other commercial cultivars. In addition, several long-day onion cultivars, 'El Capitan', 'Peckham Yel-

low Sweet Spanish' (YSS), and 'Tucker YSS', were used in the development of these three entries. Some long-day onion cultivars have high levels of FBR resistance. It is unknown what level of FBR resistance is found in 'El Capitan', 'Peckham YSS', and 'Tucker YSS'. However, if one of these cultivars is FBR resistant, that resistance could have been inherited by the NMSU entries. These long-day cultivars were not used in the development of NMSU 00-28-1, 'NuMex Casper', and 'NuMex Snowball'. This difference might partially explain the difference in FBR resistance between the FBR-resistant NMSU entries and these aforementioned entries. It is unknown whether the three long-day cultivars mentioned previously were used in the development of the commercial cultivars used in this study. In addition to the three long-day cultivars, PI 249538, a plant introduction accession from Greece, was used in the development of the three NMSU entries. In a screening of long-day onion accessions for FBR resistance, PI 249538 was found to be segregating in its resistance to FBR, with most of the bulbs being susceptible (U.S. Dept. of Agriculture, 2005). It is possible that some bulbs within this accession were resistant and that resistance was inherited within the NMSU entries. This accession was not used in the development of the other NMSU entries tested in this study. This difference might partially explain the difference in FBR resistance between the three aforementioned NMSU entries and the other NMSU entries tested.

CONCLUSION. Entries should be evaluated across years for FBR resistance. Disease severity and incidence can vary from year to year. These yearly differences make breeding for resistance difficult to achieve. In addition, bulbs should be stored for a length of time to allow the disease to develop; severity and incidence continue to increase after bulbs are stored. The storage of bulbs for 4 weeks would allow for disease development and would help improve selection efficiency for resistant bulbs. This would ensure a proper evaluation of FBR resistance and help in the development of resistant cultivars. Of the entries tested, NMSU 99-30, 'NuMex Arthur', and 'NuMex Jose Fernandez' showed the lowest disease severity and incidence in both years at harvest and after 4

Table 3. Fusarium basal rot incidence after 4 weeks of storage averaged over years (2001 and 2002) when onion entries were grown in Las Cruces, N.M.

Entry	Mean incidence at 4 weeks (%) ^z
Alabaster	57.9
Aspen	60.8
Caballero	49.4
Candy	40.0
Cimarron	51.4
Dawn	46.4
Frosty	61.9
Mesquite	58.1
NMSU 00-28-1	52.1
NMSU 99-30	20.0
NMSU 99-32	43.3
NMSU 99-33-1	42.9
NuMex Arthur	33.8
NuMex Bolo	36.7
NuMex Casper	41.9
NuMex Jose Fernandez	29.4
NuMex Snowball	45.0
Riviera	63.8
Sierra Blanca	58.6
Tara	57.5
Tequila	46.9
Utopia	41.3
Mean	47.1
LSD (5%)	13.6

^zPercentage of 20 bulbs (four replicates per entry per year) with fusarium basal rot after 4 weeks in storage.

weeks of storage, and would be logical choices to use in the development of FBR-resistant cultivars. In addition, these cultivars could be grown in fields that produce a high incidence of FBR-infected bulbs without the bulb loss that might be seen with more FBR-susceptible cultivars.

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