

# Evaluation of Onion Cultivars for Resistance to *Aspergillus niger*, the Causal Agent of Black Mold

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**ABSTRACT.** Black mold (BM), caused by *Aspergillus niger* Tiegh., is a common onion (*Allium cepa* L.) postharvest disease under hot and humid storage conditions. This study aimed to develop a BM screening protocol, to evaluate onion cultivars for resistance. The effects of pathogen isolate, inoculum density, incubation temperature, and inoculation method on severity of BM were investigated in designing the screening protocol. Variation in virulence was observed and two highly virulent subcultures, An4-2 and An39-3, were selected for study. Disease severity, both in lesion size and sporulation degree, increased with higher inoculum densities and incubation temperatures. In contrast, small lesions and no sporulation were observed when onion scales were inoculated with  $1 \times 10^4$  spores/mL or incubated at temperatures lower than 20 °C. Inoculation of onion scales or entire bulbs were compared and scale inoculation correlated better with BM incidence in storage. The procedure for BM screening protocol included: wound inoculation of onion slices with 40  $\mu$ L inoculum suspension of  $1 \times 10^5$  spores/mL, incubation at 25 °C with 12 hour photoperiod, and rating lesion diameter and sporulation 4 days after inoculation. Using this screening protocol, 42 onion cultivars were evaluated and cultivars with better storability such as 'Red Pinoy', 'Serrana', 'Dehydrator No.3', and 'Moonlight' were tolerant to *A. niger*. These cultivars showed small lesions and no sporulation during screening. On the other hand, cultivars with poor storability like 'Texas Early Grano 502', 'Granex 429', and 'Explorer' exhibited larger lesions with profuse sporulation. Lesion diameter caused by the scale inoculation correlated significantly with bulb storage loss ( $r = 0.51, P < 0.01$ ) as well as with bulb dry matter ( $r = -0.48, P < 0.01$ ) and total soluble solids ( $r = -0.51, P < 0.01$ ). The screening protocol and tolerant materials are now successfully used in the Asian Vegetable Research and Development Center onion breeding program for resistance to black mold.

Black mold (BM), caused by *Aspergillus niger* Tiegh., is a common onion (*Allium cepa*) postharvest disease under hot and humid storage conditions. Infected bulbs show black discoloration on the neck, shallow lesions on the outer scales, and streaks of black mycelia and spores beneath the outer dry scales. Severe incidences of BM on onion bulbs in storage have been reported from India (Gupta et al., 1991; Maheshwari, 1988), Sudan (Hayden et al., 1994a), Israel (Grinstein et al., 1992), Egypt (Zohri et al., 1992), Australia (Salvestrin and Letham, 1994), Taiwan (AVRDC, 1999), and the United States (Ceponis, 1986). Losses can be as high as 60% during summer storage (Tanaka, 1991). Our previous study also indicated that BM was the major disease during storage under ambient conditions in the tropics (Ko et al., 2002).

*Aspergillus niger* is generally considered a saprophyte and its spores are commonly present in air and soil (Hayden et al., 1994b).

Infection of onion bulbs usually occurs through wounds in the neck, bruised outer scales, and basal stem plates (Sumner, 1995). Spores can germinate within 3 to 6 h under high humidity, but germination is inhibited below 76% relative humidity (RH) (Sumner, 1995). Sporulation can take place 24 h after infection (Salvestrin and Letham, 1994). The optimum growth temperature of *A. niger* ranges from 28 to 34 °C, and growth is inhibited below 17 °C and above 47 °C (Sumner, 1995). Thus, BM is prevalent when onions are stored under ambient high temperatures (>30 °C) and humidity [>80% relative humidity (RH)] (Musa et al., 1973).

Chemical and cold treatments can effectively control onion BM. Bulb treatment with a mixture of diethofencarb and carbendazim, thiabendazole or imazalil (Grinstein et al., 1992) or fumigation with sulfur dioxide (Thamizharasi and Narasimham, 1993) can control BM during storage. However, chemical treatment of bulbs is undesirable due to the potential health hazards. A simple and effective control method for BM is storage at 0 to 2 °C in a cold storage facility (Tanaka et al., 1985). However, such facilities are usually not affordable to small farmers in developing countries and a more affordable control method is needed.

One promising solution is to utilize host resistance. Although no BM resistant cultivar is available, genetic variation in the degree of susceptibility to BM is present in onion. Our previous study also indicated that BM incidence varied from 10.8% to 50.8% among 12

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cultivars after a 3-month storage period under ambient conditions (23.8 to 32 °C and 67% to 98% RH). Cultivars 'Red Pinoy' and 'Serrana' were the most tolerant to BM with 10.8% and 16% disease incidence, respectively. Moreover, breeding for BM resistance is possible based on a broad-sense heritability of 0.64 estimated in our previous study (Ko, 2001).

A reliable evaluation procedure for selection of BM resistance is required, but such a procedure is not available. The objectives of this study were to develop a reliable bioassay method for evaluating resistance to *A. niger* in onion and to evaluate onion cultivars for resistance to BM.

## Materials and Methods

**INOCULUM.** *Aspergillus niger* isolates An4 and An39 were obtained from onion bulbs showing BM symptoms in the field and in a storage room of the Asian Vegetable Research and Development Center (AVRDC). Their pathogenicity was confirmed on onion bulb scales following Koch's postulates. A total of seven single-spore subcultures were derived from each isolate and stored in silica gel for long-term preservation following a modified method of Doss et al. (1984). Spores from 4-d-old potato dextrose agar (PDA, Difco Laboratory, Sparks, Md.) cultures were transferred to 7% (w/v) autoclaved skim milk. Desiccated silica gel was dispensed into screw cap vials (diam = 16 mm; height = 62 mm), half filling them. About 0.25 mL of the spore suspension was uniformly dispensed onto the silica gel. The vial was then capped, shaken on a vortex mixer, sealed with paraffin and refrigerated at 4 °C. For storage of up to 2 weeks, isolates were maintained on PDA slant tubes at 28 °C with a 12 h photoperiod.

To recover inoculum from long-term storage cultures, four spore-coated particles of silica gel were transferred to PDA and incubated at 28 °C with a 12 h photoperiod for 4 d. The cultures were flooded with sterile water and spores were harvested by gently scraping with a slide. The fungal suspension was filtered through two layers of cheesecloth to remove hyphae and conidiophores. One drop of Tween 20 was added to the suspension and vortexed for 1 min. The concentration of spores was determined with a hemacytometer and then adjusted to  $1 \times 10^5$  spores/mL or according to the need of each experiment.

To select highly virulent isolates, the 14 single-spore subcultures of An4 and An39 were inoculated onto tissues of 'Texas Early Grano 502' (TG502) onions. Bulbs were stripped of the outer dry scales and surface disinfested with 70% ethyl alcohol. Rectangular tissue slices (2 × 4 cm) were cut from the third scale layer of the bulb under aseptic conditions. Seven slices were placed in a 15-cm-diameter sterile petri dish containing two layers of filter paper moistened with 5 mL of sterile distilled water. A small wound (≈0.25 mm wide and 1.5 mm deep) was made in the middle of each onion slice with a needle, in which 40 µL of  $1 \times 10^5$  spores/mL was dispensed. After inoculation, petri dishes were sealed with paraffin and incubated at 28 °C with a 12 h photoperiod. A randomized complete block design (RCBD) was used with two replications. Symptoms were evaluated 4 d after inoculation (DAI). Lesion diameter in each slice was measured using a digital caliper (Mitutoyo 500-321, Japan). Sporulation and density on each slice was visually scored from 0 to 7, where 0 = no visible sporulation, 1 = slight sporulation with less than  $5 \times 10^5$  spores/slice, 3 = moderate sporulation with  $5 \times 10^5$  to  $5 \times 10^6$  spores/slice, 5 = heavy sporulation with  $5 \times 10^6$  to  $1 \times 10^7$  spores/slice, and 7 = very heavy sporulation with more than  $1 \times 10^7$  spores/slice.

### EFFECT OF INOCULUM DENSITY AND INCUBATION TEMPERATURE.

Two highly virulent subcultures, An4-2 and An39-3, were chosen and four inoculum densities,  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ , and  $1 \times 10^7$  spores/mL were evaluated. 'Granex 429' onion slices were inoculated with 40 µL of inoculum as described above and then incubated at 28 °C and 12 h photoperiod. The experimental design was RCBD with three replications. The effects of temperature on lesion diameter and sporulation were evaluated by preparing 'Granex 429' onion slices as described previously and incubating three petri dishes per subculture at 15, 20, 25, 30, and 35 °C with a 12-h photoperiod. A RCBD experiment design was used with three replications. The above two experiments were repeated twice.

**SCALE AND BULB INOCULATION METHODS.** Two inoculation methods were compared by inoculating *A. niger* subculture An4-2 on scales and bulbs. Seven cultivars showing different degrees of BM incidence in storage trials were used in this study. For scale inoculation, the method described previously was followed. Lesion diameter and sporulation were recorded 4 d after inoculation. For bulb inoculations, bulbs were stripped of the outer dry scales and surface sterilized with 70% ethyl alcohol. Two wounds were made 3 cm deep on both sides (symmetry) of the top portion of the bulb using a 1 mL pipette tip. A total of 40 µL of a spore suspension ( $5 \times 10^5$  spores/mL) was injected into the cavity of the wound. The inoculated bulbs were kept in a sealed plastic bag containing moist cotton to retain humidity. Each cultivar had three replications with four bulbs per replication. Bulbs were incubated at 25 °C with a 12 h photoperiod and disease development was assessed 7 d after inoculation. Bulbs were cut through the inoculated site and the severity of bulb infection was visually estimated using a score of 0 to 5 based on lesion expansion, where 0 = no symptoms, 1 = expansion <5 mm, 2 = expansion 5 to 9 mm, 3 = expansion 10 to 14 mm, 4 = expansion 15 to 19 mm, and 5 = expansion ≥20 mm with severe water soaking and profuse sporulation.

**EVALUATION OF ONION CULTIVARS FOR RESISTANCE TO *A. niger* AND RELATIONSHIP OF RESISTANCE WITH OTHER TRAITS.** Forty-two onion cultivars (including the seven cultivars used in the protocol development), collected by the Genetic Resources and Seed unit and the Bulb Allium unit, were used in this experiment. Onion cultivars were planted in an AVRDC field in November 1996, harvested in April 1997, and stored at 4 °C. Three bulbs per cultivar were randomly selected and each bulb represented a replication. The third scale was sampled centripetally from the outer layer and seven slices were prepared for artificial inoculation with subculture An4-2 as described previously. For the control treatment, a wound was made on seven slices from each cultivar and inoculated with sterile water. Disease development was assessed 2, 4, and 6 d after inoculation, but data of 4 DAI only are presented.

Bulb traits evaluated at harvest included average bulb weight, bulb color, dry matter content (DM), total soluble solids (TSS), and pyruvic acid content (following the procedures described by Schwimmer and Weston, 1961). For evaluating storability, 30 healthy uniform bulbs of each cultivar in each replication were placed separately in a 36 × 60-cm nylon bag. Onion cultivars were arranged in RCBD with two replications. Bulbs were stored for 3 months from May to July 1997 under ambient conditions. Mean maximum/minimum air temperatures were  $31 \pm 2.1/23.1 \pm 2.4$  °C and mean maximum/minimum relative humidity were  $86.8 \pm 5.9/64.3 \pm 6.4\%$ , respectively. Bulbs were examined at 3-week intervals. Storage losses were determined as the accumulated number of spoiled bulbs/total bulbs × 100.

**DATA ANALYSIS.** All percentage data were transformed by arcsine square root before analysis. Treatment means were separated by the least significant difference test (LSD) at the 5% level of significance.

Treatment effects of inoculum density and temperature on the infection of *A. niger* were statistically analyzed by the general linear model (GLM) and contrast (SAS Institute Inc., 1993). Pearson correlation coefficients between lesion size, sporulation, storage loss, and other bulb characteristics were computed with cultivar means,  $n = 42$ , using Proc Corr of Statistical Analysis System (SAS Institute Inc., 1993).

## Results

**VIRULENCE TEST.** All of the single-spore subcultures of *A. niger* caused 100% disease incidence on onion slices 4 d after inoculation. Lesion diameters ranged from 2.8 to 5.2 mm and averaged 4.7 and 3.9 mm for all subcultures of An4 and An39, respectively. Subcultures An4-2 and An39-3 were the most virulent, producing lesion diameters of 5.2 and 4.8 mm respectively. These two subcultures were selected for further study.

**EFFECT OF INOCULUM DENSITY AND INCUBATION TEMPERATURE.** Symptom severity caused by An4-2 and An39-3 on 'Granex 429' was significantly affected by inoculum density (Fig. 1). Disease development increased with increasing inoculum density. Differences in lesion diameter between subcultures and the interaction of inoculum density by subculture were not significant. Lesion diameter varied from 3.1 to 10.4 mm and sporulation score varied from

0 to 5.2 among treatments. Moderate to heavy sporulation was observed when the inoculum densities were  $10^6$  to  $10^7$  spores/mL (Fig. 1). Spores and new infections commonly were found on the edge of onion slices for treatment with  $\geq 10^6$  spores/mL, which interfered with accurate evaluation. Therefore, an inoculum density of  $10^5$  spores/mL was used for further study.

Differences in lesion diameter between subcultures, incubation temperatures and the interaction between subcultures and temperature were significant (Fig. 2). In general, subculture An4-2 caused larger lesions compared to An39-3, but differences in sporulation between these two subcultures were not significant. Lesion diameter increased with increasing incubation temperature for both subcultures. Only mild symptoms were observed when temperatures were  $<25^\circ\text{C}$ . At  $25^\circ\text{C}$ , subcultures An4-2 and An39-3 produced lesions of 5.3 mm and 3.1 mm lesion, respectively and generated only a few spores. When temperature increased to 30 or  $35^\circ\text{C}$ , *A. niger* produced dense spores on onion tissue and spores often spread establishing new colonies on onion slices which caused rating problems. Therefore, an incubation temperature between 25 to  $30^\circ\text{C}$  appeared to be optimal for rating *A. niger* infection on onion tissue.

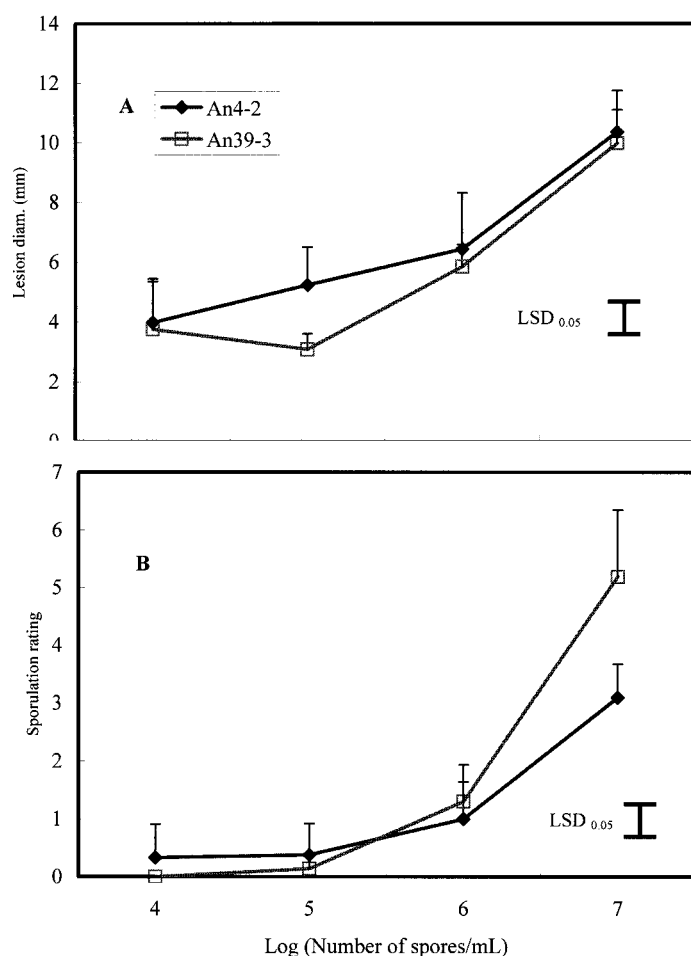


Fig. 1. Effect of inoculum density of *Aspergillus niger* subculture An4-2 and An39-3 on lesion diameter (A) and sporulation (B) on tissue of 'Granex 429' onion. Inoculated tissues were incubated for 4 d at  $28^\circ\text{C}$  with a 12 h photoperiod. Sporulation was scored from 0 (no visible sporulation) to 7 (very heavy sporulation).  $\text{LSD}_{0.05}$  for lesion diameter and sporulation was 1.54 and 0.79, respectively.

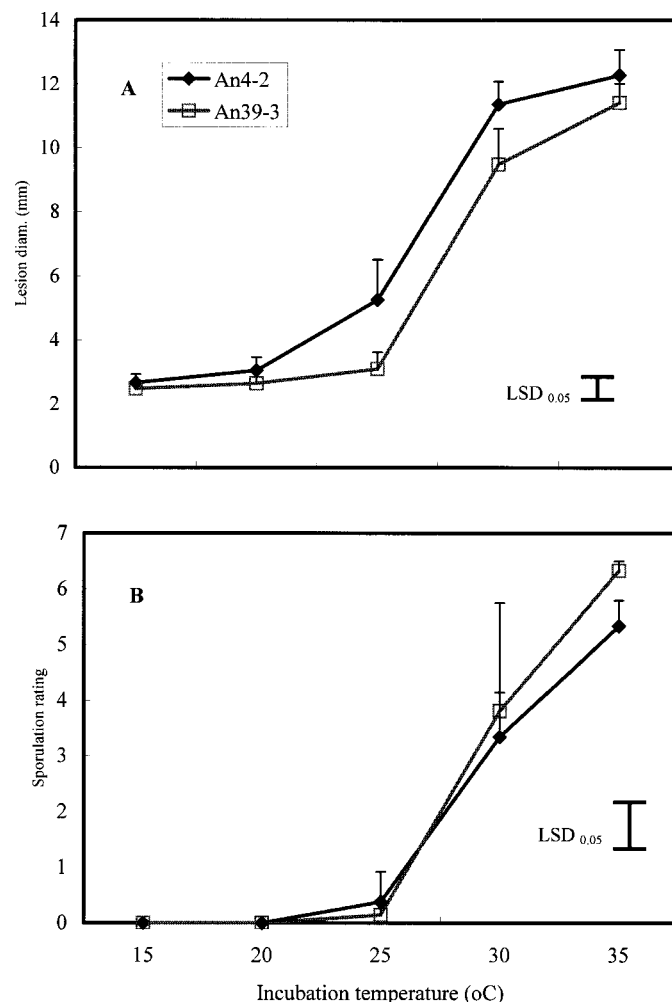


Fig. 2. Effect of incubation temperature on lesion diameter (A) and sporulation (B) caused by *Aspergillus niger* subculture An4-2 and An39-3 on tissue of 'Granex 429' onion. Inoculated tissues were incubated for 4 d with a 12 h photoperiod. Sporulation was scored from 0 (no visible sporulation) to 7 (very heavy sporulation).  $\text{LSD}_{0.05}$  for lesion diameter and sporulation was 0.72 mm and 0.9, respectively.

**SCALE AND BULB INOCULATION METHODS.** Variation in symptom severity caused by subculture An4-2 was observed for both inoculation methods on seven onion cultivars (Table 1). Lesion diameters varied from 2.2 mm ('Dehydrator No. 3') to 5.4 mm ('Texas Early Grano 502') and sporulation scores ranged from 0 ('Red Pinoy') to 2 ('Granex 429') at 4 DAI for the scale inoculation method. Infection was lowest in 'Serrana' (16.7%) and highest in 'Granex 429' (91.7%) when the bulb inoculation method was used. Bulb disease severity scores varied from 0.75 ('Serrana') to 3.13 ('Early Y Premium') (Table 1). Dry wounds were observed on tolerant cultivars such as 'Serrana' and 'Red Pinoy', while water soaking and profuse sporulation were noted on susceptible cultivars like 'Granex 429'.

The seven onion cultivars were evaluated for storage losses and incidence of BM in a previous study in 1996 (Ko et al., 2002). Lesion diameters and sporulation scores for the scale inoculations in the present study were positively correlated with total storage loss (both  $r = 0.82$ ,  $P < 0.05$ ) and with BM incidence during storage ( $r = 0.72$  and  $0.56$ ). Cultivars, such as 'Red Pinoy', 'Serrana', and 'Dehydrator No.3', had lower BM incidence (<16%) during storage, lower storage loss (<45%), and expressed tolerance to *A. niger* by showing smaller lesion diameter and less sporulation after artificial scale inoculation.

**EVALUATION OF ONION CULTIVARS FOR RESISTANCE TO *A. niger*.** A total of 42 onion cultivars were evaluated for resistance to *A. niger* by the scale inoculation method. Differences in *A. niger* infection among cultivars were highly significant. Four days after inoculation, lesion diameters among cultivars ranged from 1.6 mm ('XP8403') to 5.4 mm ('Texas Early Grano 502'), and sporulation scores ranged from 0 to 2 (Table 2). Cultivars such as 'Red Pinoy', 'Sweet Success', 'Red Creole', 'Mutuali IPA', 'XP8403', and 'Moonlight', with small lesion diameter (<2.5 mm) and no sporulation, were considered tolerant to *A. niger*. Lesion diameters on susceptible cultivars, 'Texas Early Grano 502' and 'Explorer', were about twice the size of tolerant cultivars. It was noted that lesions of

susceptible cultivars expanded rapidly at 6 DAI, while further disease development was minimal on tolerant cultivars.

**RELATIONSHIPS BETWEEN DISEASE SEVERITY AND BULB CHARACTERISTICS.** Bulb characteristics among the 42 cultivars were very diverse. There were yellow (28 cultivars), red (8 cultivars) and white (6 cultivars) skin colors. Bulb mass ranged from 114 g ('Local Red') to 420 g ('Redbone'). Storage losses ranged from 39% ('Moonlight') to 97.4% ('Torrens White'). In terms of chemical compositions, DM varied from 5.0% ('Linda VST') to 12.7% ('Primero'); TSS ranged from 5.1% ('Linda VST') to 11.3% ('Primero'); and pyruvic acid (PA) varied from 2.6 to 9.5  $\mu\text{mol}\cdot\text{g}^{-1}$  fresh mass for 'Yellow Granex' and 'Red Creole', respectively (Table 2). Disease severity caused by the scale inoculation method was positively correlated with storage loss ( $r = 0.51$ ,  $P < 0.01$ ) but negatively correlated with DM ( $r = -0.48$ ,  $P < 0.01$ ) and TSS ( $r = -0.51$ ,  $P < 0.01$ ). Bulb size and pyruvic acid were not significantly correlated with *A. niger* infection severity (Table 2).

## Discussion

BM is a major storage disease of onion under high temperatures and high relative humidity common in the tropics and subtropics (Musa et al., 1973). Variation in virulence was observed among single-spore subcultures. *Aspergillus niger* infection lesion size after onion scale inoculation was dependent on inoculum density and temperature. Very small lesions and poor sporulation occurred when inoculum density was  $10^4$  spores/mL. The effects of inoculum density and temperature may be related to general defense mechanisms. This was shown with *Botrytis* species, where onion cell walls accumulated phenolics to resist the pathogen under low inoculum conditions (Stewart and Mansfield, 1984; Stewart and Mansfield, 1985). Lesion size increased with incubation temperature in a sigmoid manner. Lesion size increased from 25 to 30 °C but became smaller at >30 °C (Fig. 2). Such a result was similar to that described by Subbarao and Michailides (1995) who reported that temperature

Table 1. Severity of black mold caused by *Aspergillus niger* (subculture An4-2) on onion tissues, using scale- and bulb-inoculation methods, and its relationship with storability under ambient conditions.

Cultivar	Origin	Scale inoculation <sup>z</sup>		Bulb inoculation <sup>y</sup>		3-month storage <sup>x</sup>	
		Lesion diam (mm)	Sporulation score	Incidence (%)	Disease severity	Total storage loss (%)	Black mold (%)
Dehydrator No. 3	U.S.	2.2	0.1	41.7	1.1	45.0	15.0
Red Pinoy	Philippines	2.4	0.0	50.0	1.3	30.0	10.8
Serrana	U.S.	2.5	0.4	16.7	0.8	43.3	16.0
Galil	Israel	2.7	0.4	79.2	1.5	71.7	37.5
Early Y. Premium	RSA	4.0	0.8	54.2	1.8	66.5	37.9
Granex 429	U.S.	4.9	2.0	91.7	3.1	85.0	31.7
Texas E. Grano 502	U.S.	5.4	1.0	54.2	1.2	78.3	38.3
LSD <sub>(0.05)</sub>		1.7	1.3	45.0	1.1	19.9	19.9
Mean of all cultivars		3.4	0.7	55.4	1.5	60.0	26.7
Correlation with lesion diameter		---	0.82*	0.47 <sup>NS</sup>	0.54 <sup>NS</sup>	0.82*	0.72 <sup>NS</sup>
Correlation with sporulation		0.82*	---	0.63 <sup>NS</sup>	0.85*	0.82*	0.56 <sup>NS</sup>

<sup>z</sup>Onion scales were wound inoculated with 40  $\mu\text{L}$  suspension ( $10^5$  spores/mL) and incubated at 25 °C with a 12 h photoperiod for 4 d. Sporulation was scored from 0 (no visible sporulation) to 7 (very heavy sporulation).

<sup>y</sup>Bulbs were wound inoculated with 40  $\mu\text{L}$  suspension ( $10^5$  spores/mL) and incubated at 25 °C with a 12 h photoperiod for 7 d. Disease severity on bulbs was categorized on a 0 to 5 scale, where 0 = no symptoms, 1 = lesion expansion <5 mm, 2 = expansion 5 to 9 mm, 3 = expansion 10 to 14 mm, 4 = expansion 15 to 19 mm, and 5 = expansion  $\geq 20$  mm with severe water soaking and profuse sporulation.

<sup>x</sup>Data were used from Ko (2001). Mean percent of storage loss and black mold incidence rated at 3 months after storage. All percentage data were transformed by arcsine square root before analysis. During storage in 1996 the mean maximum/minimum air temperatures were  $31.9 \pm 2.2/23.8 \pm 1.2$  °C and mean maximum/minimum relative humidity and were  $97.6 \pm 1.3/66.6 \pm 7.5\%$ , respectively.

<sup>NS,\*</sup>Nonsignificant or significant at  $P = 0.05$ , respectively.

influenced *A. niger*'s incubation and latent periods, lesion size, rate of lesion expansion, and sporulation. Suppression of lesion expansion at low temperature may be associated with low amounts of certain virulence factors. Sellam et al. (1977) reported that production of pectolytic and cellulolytic enzymes, common virulence factors in several onion pathogens, was suppressed under low temperatures.

*Aspergillus niger* is also a wound parasite, similar to *Botrytis allii*, the causal agent of onion neck rot. Thus, artificial wounding was required when inoculating *A. niger* either on scales or bulbs. Disease severity induced by the bulb inoculation method was positively correlated with lesion diameter induced by the scale inoculation method. However, the scale inoculation method has several advantages as an evaluation method. It is simple, easy to

Table 2. Disease severity caused by *Aspergillus niger* (subculture An4-2) using the scale inoculation method on 42 onion cultivars and its relationship with storability and bulb characteristics.

Cultivar <sup>z</sup>	Origin	Black mold severity			Bulb characteristics <sup>y</sup>				
		Lesion diam (mm)	Sporulation score <sup>x</sup>	Storage loss (%)	Color	Avg mass (g)	DM (%)	TSS (%)	Pyruvic acid (μmol·g <sup>-1</sup> FW)
XP8403 (OP)	U.S.	1.6	0.0	67.5	Yellow	329	8.5	8.6	6.2
Red Creole (OP)	Denmark	1.8	0.0	44.0	Red	152	11.7	10.5	9.5
Moonlight/ RS533 (H)	Netherlands	2.0	0.0	39.0	White	345	7.0	7.5	5.0
Dehydrator No. 3	U.S.	2.2	0.1	61.7	White	162	11.0	10.3	5.2
Mutuali IPA	Brazil	2.3	0.0	91.7	Red	135	9.1	8.7	6.3
Red Pinoy (OP)	Philippines	2.4	0.0	45.1	Red	149	11.6	11.2	6.0
Sweet Success (H)	U.S.	2.4	0.0	59.5	Yellow	348	6.1	6.4	3.9
Yohzuiki	Japan	2.5	0.1	80.0	Yellow	322	5.4	6.3	4.9
Serrana (OP)	U.S.	2.5	0.4	55.5	Yellow	221	8.1	8.4	5.8
SSC6060 (H)	U.S.	2.5	0.3	69.0	Yellow	357	5.3	5.3	3.8
Primero	U.S.	2.6	0.1	68.3	White	204	12.7	11.3	4.4
Burgundy	U.S.	2.6	0.1	92.9	Red	327	6.1	6.6	5.8
Galil (H)	Israel	2.7	0.4	62.9	Yellow	264	5.4	6.1	4.6
Yellow Granex F1	Denmark	2.8	0.5	75.0	Yellow	293	5.8	6.3	2.6
BGS 85 F1	Netherlands	2.8	0.3	78.0	Yellow	405	5.1	5.2	5.3
Hatig de Valence	France	2.8	0.1	76.2	Yellow	ND	6.1	6.2	3.9
PS13489	U.S.	2.9	0.1	72.0	Yellow	384	5.5	5.5	3.8
Linda VST/ PSX8589	U.S.	3.0	0.5	91.0	Yellow	359	5.0	5.1	3.8
Super High Gold No.1	Japan	3.1	0.0	82.6	Yellow	400	5.2	6.0	7.3
Local Red	India	3.1	0.0	70.0	Red	114	ND	ND	ND
Stetson (H)	U.S.	3.2	0.5	75.0	White	277	6.8	6.5	5.6
Early Supreme	U.S.	3.3	0.4	95.3	White	265	6.7	6.8	4.7
Mercedes	U.S.	3.3	0.1	83.0	Yellow	283	8.6	8.6	5.7
Texano	Denmark	3.4	0.5	46.2	Yellow	186	ND	ND	ND
Ringer Grano Improved	U.S.	3.6	0.0	91.7	Yellow	327	5.6	5.7	4.6
Redbone	U.S.	3.7	0.6	57.0	Red	420	ND	ND	ND
RS209	Netherlands	3.7	1.5	50.0	Yellow	178	5.9	5.8	4.7
Red Granex (H)	U.S.	3.8	1.2	91.0	Red	274	6.6	7.4	5.9
Marquis (H)	U.S.	3.8	0.5	66.0	Yellow	373	5.3	5.4	4.3
XPH 6074 (H)	U.S.	4.0	0.4	85.7	Red	126	ND	ND	ND
Early Yellow Premium	RSA	4.0	0.8	76.0	Yellow	198	6.2	6.4	5.5
Mercedes	U.S.	4.1	0.6	89.5	Yellow	337	7.1	7.1	8.1
Gran Prix	RSA	4.1	0.1	73.0	Yellow	372	6.2	6.1	4.6
Sunex1502 (H)	U.S.	4.1	0.3	65.0	Yellow	341	6.5	6.5	3.4
Rio Zorro (H)	U.S.	4.4	1.2	96.1	Yellow	362	5.2	5.3	3.2
Arad (H)	Israel	4.4	1.3	87.0	Yellow	244	6.2	6.7	4.7
Payola (H)	U.S.	4.6	1.0	85.7	Yellow	341	6.2	5.8	3.6
Granex (H)	Israel	4.6	0.4	72.0	Yellow	301	5.7	5.7	4.0
Torrens White (H)	Australia	4.7	0.8	97.4	White	223	6.1	6.7	6.2
Granex 429 (H)	U.S.	4.9	2.0	83.0	Yellow	302	5.5	5.9	4.0
Explorer (H)	Netherlands	5.3	1.9	96.3	Yellow	373	5.8	6.1	4.3
Texas Early Grano 502 (OP)	RSA	5.4	1.0	93.0	Yellow	264	5.1	5.4	5.2
Correlation with lesion diameter		1.00	0.74**	0.51**	0.05 <sup>NS</sup>	0.16 <sup>NS</sup>	-0.48**	-0.51**	-0.27 <sup>NS</sup>

<sup>z</sup>OP = an open pollinated cultivar; H = a hybrid cultivar.

<sup>y</sup>Bulb characteristics were evaluated at harvest. DM = dry matter content; TSS = total soluble solids; ND = not determined due to lack of sample.

<sup>x</sup>Sporulation was scored from 0 (no visible sporulation) to 7 (very heavy sporulation); FW = fresh weight.

<sup>NS,\*\*</sup>Nonsignificant or significant at  $P = 0.01$ , respectively. Data are the means of three replications in each cultivar for black mold severity and two replications for storability and bulb characteristics.

apply, and requires less space and plant material. More importantly, it produces more consistent and reliable results due to the ease of symptom recording method. Both lesion diameter and sporulation score were correlated with total storage loss (both  $r=0.82$ ,  $P<0.05$ ) (Table 1). The insignificant correlation of BM with lesion diameter and sporulation score could be due to the small sample size ( $n=7$ ) in this experiment. In assessing sporulation, score is less time consuming, and can be applied when evaluating large number of cultivars. In general, the scale inoculation method developed in this study, is quite reliable and efficient. This will greatly enhance selection and breeding for resistance to BM in the future.

A total of 42 cultivars were evaluated for resistance to *A. niger* by the scale inoculation method. No immune cultivars were found but several tolerant cultivars were identified, including white, yellow, and red skinned onions. Contradictory reports were found regarding the relationship between skin color and tolerance to BM (Rao and Rajasab, 1992; Sumner, 1995). Our study indicated that resistance to black mold is not associated with bulb color, which was in agreement with our previous study (Ko et al., 2002). Variation among cultivars for susceptibility to *A. niger* may be more related to high DM and TSS contents. Results from our previous study (Ko et al., 2002) suggested that cultivars that had higher DM tended to have less storage loss ( $r=-0.60$ ,  $P<0.01$ ) and lower BM incidence ( $r=0.68$ ,  $P<0.01$ ). In the present study, lesion diameter was negatively correlated with DM ( $r=-0.48$ ,  $P<0.01$ ) and TSS ( $r=-0.51$ ,  $P<0.01$ ) (Table 2). Marin et al. (1998) indicated that *A. niger* spore germination was very rapid at high moisture ( $>0.90$  water availability) with an almost linear increase with time. It is likely that tissues of high DM cultivars have low water content which are less favorable for the growth of microorganisms and resulting in poor disease development. The moderate correlation coefficients suggest the presence of other factors associated with the resistance to BM. Tanaka (1991) reported that when onion was infected by *A. niger*, the concentration of citric and lactic acids increased in the infected tissues.

This is the first report on the development and use of a bioassay for evaluating onion cultivars resistant to *A. niger*. Several cultivars tolerant to *A. niger* were identified. A breeding program has been initiated using both the screening method and tolerant cultivars. DM and TSS were shown to be moderately correlated with tolerance to *A. niger*, but further studies are needed to gain a better understanding on the resistance mechanism to *A. niger* in onion.

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