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Reduced Iris Yellow Spot Symptoms through Selection within Onion Breeding Lines

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Abstract: Iris yellow spot (IYS) disease in onion (*Allium cepa* L.) is caused by onion thrips (*Thrips tabaci* L.) vectored *Iris yellow spot virus* (IYSV). The absence of cultivars that are resistant/tolerant to thrips and/or IYS is a challenge for onion bulb and seed production worldwide. To measure selection progress for reduced/delayed IYS symptom expression in onion breeding lines after two selection cycles, selections were performed in 2011 on previously evaluated lines that exhibited a reduced symptom expression after one selection cycle. Selected plants from each line were massed in a cage and the resulted progenies were evaluated in 2013 and 2014 along with their original populations and a susceptible check—'Rumba'. In some comparisons, the selection progress for delayed/reduced IYS symptom expression was observed for some breeding lines. Plants of most selected breeding lines exhibited less disease expression than plants of 'Rumba'. For some selections, a low disease severity was observed even with a relatively high number of thrips per plant. These results suggest that further improvement might be achievable with additional cycles of selection.

Keywords: *Allium cepa*; *Iris yellow spot virus*; *Thrips tabaci*

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1. Introduction

Iris yellow spot virus (IYSV) (family *Bunyaviridae*, genus *Tospovirus*) and Iris yellow spot (IYS) disease occurrence have been reported in several onion (*Allium cepa* L.) growing regions of the world including several US states since first reported as the *Tomato spotted wilt virus* in southern Brazil [1]. In the US, IYS has been confirmed in most of the onion producing states [1]. Apart from onions, IYSV has also been reported on other species of the genus *Allium* such as, *A. altaicum*, *A. galanthum*, *A. roylei*, *A. schoenoprasum*, *A. tuberosum*, *A. vavilovii* [2] and on *A. sativum* [3]. In addition, IYSV can also naturally infect more than 50 plant species [1].

Onion thrips (*Thrips tabaci*) is the main vector to transmit IYSV. Although tobacco thrips (*Frankliniella fusca*) can act as a vector of IYSV, their transmission efficiency is considerably lower as compared to onion thrips [4]. At its larval stage, onion thrips acquire the virus after feeding on infected plants, then the virus multiplies inside them, and while feeding on healthy leaf tissue, they can transmit the virus [4–6]. Onion transplants and infected volunteer plants can act as potential sources of IYSV inoculum; hence, onion thrips can spread IYSV to healthy onion plants after feeding on these plants [1]. IYS-infected onion plants initially produce small necrotic, irregular or diamond-shaped straw-colored lesions on their leaves, which later grow and coalesce into larger lesions that can encompass an entire leaf [1]. This decrease in leaf photosynthetic area eventually affects bulb yield by decreasing the number of larger-sized bulbs that often have a higher market value than smaller bulbs [7–11]. In onion seed crops, yields can be substantially reduced due to girdling and lodging of seed scapes when the number and sizes of lesions increase [8].

An integrated approach, such as, virus free transplants, crop rotation with nonhosts, reduced plant stress, modified cultural practices, control of vectors with insecticides and host–plant resistance is required to effectively control thrips infestation and spread of IYS in onions [1]. However, each approach to control onion thrips and IYS has its limitations. For instance, dry and hot (>30 °C) climatic conditions can accelerate thrips buildup and can amplify the spread of IYS disease due to the limited efficacy of insecticidal applications to control thrips under these conditions [12]. In addition, high costs, ecological and environmental impacts and an increase in thrips resistance to insecticides limit the options for effective control of onion thrips and IYS [1,12–14]. Though differences for thrips number, thrips feeding injury and IYS disease development have been reported in onion germplasm, still no onion cultivar with complete resistance to thrips and/or IYS has been found or developed [1,7,9,11,12,14–17].

In several studies, differences in leaf epicuticular waxes and leaf color among cultivars likely affected thrips preference and feeding injury [18–21]. In addition, greater thrips attraction to bluish foliage cultivars as compared to cultivars with greenish foliage has been reported [12,22]. Lighter green foliage with a glossy appearance that accumulates less epicuticular wax seems to hinder thrips growth [20] with a decrease in egg-hatching [1]. Differences were reported in thrips feeding injury and yield among cultivars when thrips infestation levels were similar [23].

Differences among onion cultivars for IYS incidence and severity have been observed in the states of Colorado [24], New Mexico [12,14,16,25,26], New York [27], Oregon [28], and Washington [7]. In New Mexico, breeding line New Mexico State University (NMSU) 05-33-1 showed fewer IYS symptoms among 13 winter-sown onion entries [14]; plants of 'Cometa' and NMSU 05-35-1 were more tolerant to IYS and also had lower levels of IYSV than other entries [26] and selected breeding lines, NMSU 10-575-1, 10-577-1, and 10-582-1 exhibited less IYS severity than their original populations that were derived from plant introduction accessions [16]. In an earlier work, selected breeding lines, NMSU 10-776, 10-782, 10-785, 10-807, and 10-813 had less severe disease and a lower disease incidence than their respective original breeding populations [29]. These original populations were intermediate-day onion breeding lines derived from crosses made between Grano and Sweet Spanish germplasm. When 75 different plant introduction (PI) accessions were evaluated, plants of PIs 239633 and 546192 exhibited fewer IYS disease symptoms than plants of most accessions [12]. Onion breeding lines NMSU 14-281, 14-208, and 14-240 evaluated at the Malheur Experiment Station in Oregon exhibited less IYS damage than three commercial cultivars [28].

This study builds upon earlier work [29], in which some first-generation breeding lines performed better than their original population for reduced IYS symptom expression and incidence. The main objective of this study was to determine if selected onion breeding lines exhibited reduced IYS symptom expression after two selection cycles when compared to their original populations from the NMSU onion breeding program. Since IYS symptom development is a function of thrips feeding [23,30], the same breeding lines were evaluated for thrips number per plant to determine differences between selected breeding lines and their original populations.

2. Materials and Methods

2.1. Plant Material

In 2011, individual onion plants with reduced IYS symptoms were selected from several populations (Figure 1) [29]. The original populations used for selection were intermediate-day breeding lines derived from crosses between lines/cultivars which belong to Grano and Sweet Spanish types of onion germplasm. Selection progress of the selected germplasm towards thrips and IYS resistance was measured in 2011 and 2012 [29,31]. At harvest, individual plants with reduced IYS symptoms were selected again from plots of improved populations (first generation). In 2012, seeds from these selected plants were produced by massing selected bulbs separately from each population in insect-proof cages (three bulbs per cage) to produce the second selected generation. In 2013, 16 entries comprised of 10 selected breeding lines and 6 original populations, and a susceptible check cultivar, 'Rumba' (Nunhems USA, Parma, ID, USA), were evaluated to measure the selection progress for reduced IYS symptoms. In 2014, only 5 selected breeding lines, 3 original populations, and 'Rumba' were evaluated due to the lack of seed for some of the entries tested in 2013.

Figure 1. An example of selected plants (left of orange stake) as compared to unselected plants (right of orange stake). Plants were visually selected on the basis of less thrips damage, full bulb maturity, and mainly fewer IYS symptoms. Plants were from New Mexico State University (NMSU) breeding lines with a history of reduced Iris yellow spot (IYS).

2.2. Planting and Field Design

To ensure the presence of IYS inoculum and viruliferous onion thrips in the field, a field layout was designed in a way to bring thrips and IYSV to the field and allow for the desired spread of both throughout the field. A similar field layout design was used in several other IYS studies conducted at NMSU [12,14,16,26,29]. In the autumns of 2012 and 2013, saved bulbs from previous year's studies showing extreme IYS symptoms were planted in the study field due to the likely presence of IYSV-infected thrips within the dry scales of these bulbs. The IYS-susceptible cultivar 'NuMex Dulce' was sown in October of 2012 and 2013 after every two rows of the test plots to act as disease spreader rows. In addition, no chemical control methods were used. The presence of IYSV in plants of border and spreader rows was confirmed with ELISA [16].

All entries were seeded in black plastic flats filled with Metro Mix-360 (Sun Gro, Bellevue, WA, USA) on 7 January 2013 and 15 January 2014 in a greenhouse at the Fabian Garcia Science Center, Las Cruces, New Mexico, USA. Seedlings were transplanted in the field at the Leyendecker Plant Science Research Center in Las Cruces, New Mexico, USA (N 32°11′52.2384″; W 106°44′33.252″) on 11 March 2013 and 17 March 2014 when plants reached the 4–5 leaf stage. The lengths and widths of the plots were 3.0 m and 0.5 m, respectively, with 0.6 m alleys between two adjacent plots in the same planting bed. Each year, the field design was comprised of three replications for each entry in a randomized complete block (RCB) design. All the cultural practices to grow plants in this study were followed as per recommendations to grow onions in southern New Mexico [32]. However, no insecticides were applied. Temperature, humidity and other environmental conditions were collected by a nearby weather station [33].

2.3. Data Collection

For both years, ten plants in each plot were randomly selected and marked for subsequent data collection for IYS severity and thrips number. Thrips numbers (both adult and larvae) were recorded from these same plants in each plot at 10, 13, and 16 weeks after transplanting (WAT) in 2013 and 2014. IYS disease severity ratings were conducted at three weeks after each thrips count—i.e., 13, 16 and 19 WAT in both years. IYS disease symptom severity ratings were performed on the same ten plants from each plot at each rating time on a scale of 0 to 4, where 0 = no symptoms; 1 = 1–2 small-sized lesions per leaf; 2 => 2 medium-sized lesions; 3 = lesions coalescing on more than 25% of the leaf; 4 = more than 50% leaf death [12]. IYS disease incidence was calculated as percentage of plants from the ten plants rated for disease severity that showed IYS symptoms.

2.4. Data Analysis

Data for each year were analyzed separately due to differences in number of entries tested in each year and various environmental factors. Data were analyzed for each selected breeding line, original population, and 'Rumba' as an individual comparison for each trait. Proc General Linear Models (GLMs) statement in SAS (SAS 9.0, SAS Institute Inc., Cary, NC, USA) was used to determine if there were differences among entries for IYS severity ratings and incidence, thrips number, and leaf color and glossiness. The "Proc Means" command in SAS was used to calculate the mean of each character. For each desired comparison, the "Pdiff" command in SAS was used to test for mean differences for thrips numbers, disease severity rating, and disease incidence at $p \leq 0.05$.

3. Results and Discussion

IYS symptoms of straw-colored lesions were visible on the border and spreader row plants by the end of April or early May in each year. The presence of IYSV was confirmed in these plants with ELISA (data not presented). IYS symptoms appeared on plants of the test plots by end of May in 2013 and by the third week of June in 2014. IYS was more severe in 2013 than in 2014, and this difference might be due to the presence of conducive environmental conditions (hot and dry) during May and June in 2013 for the buildup of thrips, spread of IYSV, and IYS symptom development (Figure 2). At the last severity rating in both years, all plants rated in each plot had visible IYS symptoms; therefore, data are not presented for disease incidence at the last rating. In addition, at the time of the final severity rating in 2013, plants had started the senescence process in almost one-third of the total number of plots. Other factors, such as physiological development of plants and the ability to tolerate stress, might have influenced the visible expression of IYS in each year [12,16]. In addition, field location as well as field row lengths in each study year might also have influenced thrips movement and IYS spread in test plots. Generally, onion thrips infestation begins on the field edges [34] and seems to cause greater feeding damage on onion plants in test plots in fields with shorter rows as compared to onion fields with longer rows.

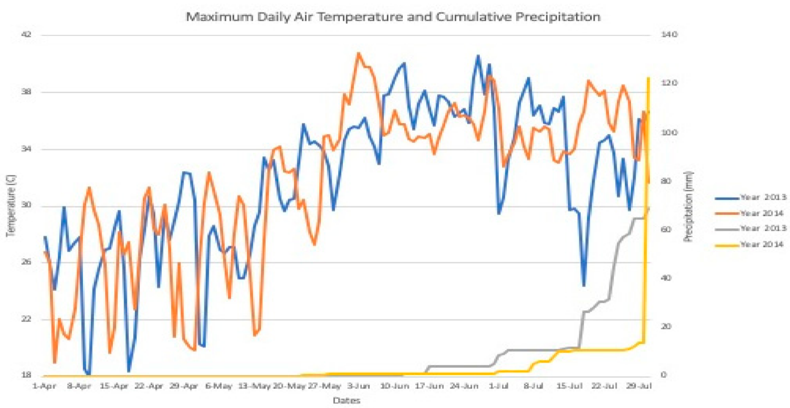

Figure 2. Maximum daily air temperature (degree C) and cumulative precipitation (mm) at Leyendecker Plant Science Research Center in Las Cruces, New Mexico, USA, from April through July of 2013 and 2014.

In both years, several instances existed in which plants of the selected breeding lines exhibited fewer and less severe IYS symptoms than plants of their original populations (Figure 3). In 2013, plants of NMSU 12-239 exhibited less IYS than plants of its original population, NMSU 07-53-1 (Table 1). However, the differences between the selected and original populations at 13 WAT were observed only at $p \leq 0.1$ level only. One possible explanation for fewer IYS symptoms on plants of NMSU 12-239 could be their slower buildup of thrips from 13 WAT to 16 WAT as compared to the plants of NMSU 07-53-1 (Table 2). At 16 weeks, plants of NMSU 12-239 had fewer thrips than plants of NMSU 07-53-1 (Table 2). With fewer thrips per plant, there would be fewer opportunities for thrips to infect plant tissue with IYSV and allow for the subsequent development of associated lesions on leaves after three weeks. In 2014, when fewer disease symptoms were observed on all plants, NMSU 12-239 produced fewer plants with disease symptoms and plants with less severe symptoms than NMSU 07-53-1 at 16 WAT (Table 1). As compared to 2013, fewer thrips were observed on plants in 2014 and as a result, plants of NMSU 12-239 had a similar number of thrips as plants of NMSU 07-53-1 at all three dates (Table 2) and subsequently thrips number was not the reason in differences in IYS disease mentioned previously.

Figure 3. From left to right, IYS symptoms on plants of susceptible check: 'Rumba' (**A**), original population NMSU 07-54-1 (**B**), and selected breeding line NMSU 12-337 (**C**) when grown in field conditions conducive for disease development.

Table 1. Mean values and comparisons of selected breeding lines with original populations for Iris yellow spot (IYS) severity rating and incidence measured several times throughout the 2013 and 2014 growing seasons at the Leyendecker Plant Science Research Center in Las Cruces, New Mexico, USA.

	IYS Disease				
	severity rating [y]			incidence (%) [x]	
	Time after transplanting (weeks)				
Entry [z]	13	16	19	13	16
	2013				
NMSU 07-32-2	1.2	2.3	3.6	90.0	100.0
NMSU 12-335	0.3 *	1.4 *	2.7 *	33.3 *	100.0
NMSU 07-52-1	1.1	1.9	2.8	90.0	100.0
NMSU 12-236	0.4 *	1.6	2.3 *	43.3 *	100.0
NMSU 07-53-1	0.8	1.8	2.9	76.7	100.0
NMSU 12-238	0.7 *	1.5	2.3	70.0	100.0
NMSU 12-239	0.5 *	1.1 *	2.2 *	60.0	100.0
NMSU 12-242	0.7	1.2 *	2.6	63.3	100.0
NMSU 12-243	0.5	1.1 *	1.6 *	53.3 +	100.0
NMSU 07-54-1	0.7	1.9	2.7	85.0	100.0
NMSU 12-337	0.4	1.2 *	1.8 *	40.0 *	100.0
NMSU 07-55-1	1.1	2.4	3.2	93.3	100.0
NMSU 12-796	0.7*	1.9 *	3.3	63.3 *	100.0
NMSU 07-56-2	1.3	2.6	3.7	93.3	100.0
NMSU 12-340	1.1	2.3 *	3.6	90.0	100.0
NMSU 12-342	0.9 *	2.1 *	3.6	83.3	100.0

Table 1. *Cont.*

	IYS Disease				
	2014				
NMSU 07-53-1	0.2	0.9	1.1	20.0	93.3
NMSU 12-239	0.1	0.5 *	1.3	13.3	53.3 *
NMSU 12-243	0.3	0.4 *	1.1	33.3	40.0 *
NMSU 07-54-1	0.0	0.8	1.2	3.3	80.0
NMSU 12-337	0.1	0.8	1.4	10.0	76.7
NMSU 07-56-2	0.3	1.0	1.4	30.0	96.7
NMSU 12-342	0.7	1.0	2.0	70.0	100.0

[z] Entries were tested in a randomized complete block design with three replications. Indentation of entries represents pedigree tree. Entry means with "*" or "+" as superscript indicate that selected breeding lines were different from the mean value of its original line at $p \leq 0.05$ or $p \leq 0.1$, respectively. [y] Ten plants per plot were rated individually for IYS severity on a scale of 0 to 4, where 0 = no symptoms, 1 = 1 to 2 small lesions per leaf, 2 => 2 medium-sized lesions per leaf, 3 = lesions coalescing on more than 25% of the leaf, and 4 = more than 50% leaf death. [x] Percentage of plants showing IYS disease symptoms were calculated based on 10 plants per plot.

Table 2. Mean values and comparisons of selected lines with original populations and susceptible check 'Rumba' for thrips number per plant in the 2013 and 2014 growing seasons at the Leyendecker Plant Science Research Center in Las Cruces, New Mexico, USA.

	Onion Thrips Per Plant [y]		
	Time after transplanting (weeks)		
Entry [z]	10	13	16
	2013		
NMSU 07-32-2	42.7	25.9	30.2
NMSU 12-335	22.4	20.2 *	26.8
NMSU 07-52-1	35.3	46.4	53.5
NMSU 12-236	21.7	34.1	44.1
NMSU 07-53-1	32.8	34.3	48.7
NMSU 12-238	20.3	30.5	34.1
NMSU 12-239	17.5	22.2 *	27.4
NMSU 12-242	21.6	31.2	33.4
NMSU 12-243	20.7	28.1	35.2
NMSU 07-54-1	21.5	15.2 *	55.7
NMSU 12-337	24.1	25.0 *	66.5
NMSU 07-55-1	38.0	33.9	41.6
NMSU 12-796	28.4	36.7	43.3
NMSU 07-56-2	29.7	32.5	12.8
NMSU 12-340	34.2	41.9	29.8
NMSU 12-342	13.5 *	21.5 *	15.6
Rumba	32.3	44.3	37.3
	2014		
NMSU 07-53-1	7.1 *	21.4 *	12.6 *
NMSU 12-238	7.5 *	18.0 *	16.7 *
NMSU 12-239	8.2 *	19.9 *	9.3 *
NMSU 12-243	6.5 *	9.8 *	18.8 *
NMSU 07-54-1	10.7 *	18.8 *	20.2 *
NMSU 12-337	10.7 *	21.4 *	15.0 *
NMSU 07-56-2	9.0 *	22.3 *	6.8 *
NMSU 12-342	8.3 *	18.8 *	8.0 *
Rumba	21.2	34.9	33.6

[z] Entries were tested in a randomized complete block design with three replications. 'Rumba' was a susceptible check in the study. Entry means with "*" as superscript indicate that entry mean was significantly different from the susceptible check 'Rumba', at $p \leq 0.05$. [y] The number of thrips (larvae and adults) per plant was counted from ten randomly selected plants in the center of each plot.

In both years, plants of NMSU 12-243 exhibited a reduced IYS symptom severity at 16 WAT when compared to plants of the original breeding line, NMSU 07-53-1 (Table 1). One possible explanation for the lower disease severity of NMSU 12-243 could be fewer thrips per plant at 13 WAT than on plants of NMSU 07-53-1 (Table 2). However, this thrips difference was only observed in 2014 and not in 2013 (Table 2). In 2013, IYS symptom development did not proceed as rapidly in plants of NMSU 12-243 as plants of NMSU 07-53-1 as evidenced by the lower disease severity at 19 WAT of 12-243 (1.6) as compared to 07-53-1 (2.9) (Table 1). In addition, disease incidence of NMSU 12-243 (53.3, 40.0) was lower than for NMSU 07-53-1 (76.7, 93.3) at 13 WAT in 2013 and 16 WAT in 2014, respectively (Table 1). The differences in disease severity and incidence observed in 2013 between NMSU 12-243 and NMSU 07-53-1 were surprising given the high thrips pressure and similar number of thrips per plant between the two entries at each observation date (Table 2). This result could suggest that thrips pressure is not the only determining factor in IYS disease severity and incidence.

In 2013, plants of selected breeding lines, NMSU 12-337 and 12-342, exhibited less severe disease symptoms when compared to plants of NMSU 07-54-1 and 07-56-2, respectively, at two of the three ratings (Table 1). These differences were not observed in 2014 most likely due to less disease development in 2014 (Table 1). For both NMSU 12-337 and 12-342, there was a three-week period during the observation time in which disease development was delayed as compared to their original populations. This result is encouraging for future breeding efforts as a delay in symptom development can lessen the impact of IYS on onion bulb yield [35]. At the first thrips count in 2013, fewer thrips were present on plants of NMSU 12-342 than on plants of NMSU 07-56-2 (Table 2), which could partially explain the lower disease severity of NMSU 12-342 plants at 13 WAT (Table 1). As alluded to earlier, thrips pressure may not be the sole determining factor for IYS disease severity. Plants of NMSU 12-342 and 07-56-2 had a similar number of thrips at 13 and 16 WAT (Table 2) even though plants of 12-342 exhibited less severe disease symptoms than plants of 07-56-2 at 16 WAT (Table 1). A similar result was observed for plants of NMSU 12-337 and 07-54-1 in which both entries exhibited a similar thrips number per plant at all observation dates (Table 2) while plants of NMSU 12-337 exhibited less severe disease symptoms at two rating dates (Table 1).

In 2013, plants of the selected breeding line, NMSU 12-335 (0.3, 1.4, 2.7), exhibited less severe disease than plants of its original population NMSU 07-32-2 (1.2, 2.3, 3.6) at 13, 16, and 19 WAT, respectively (Table 1). These two breeding lines were only tested in 2013 and not in 2014. The differences in disease severity at 13 WAT might be due to fewer thrips per plants of plants from NMSU 12-335 as compared to plants from NMSU 07-32-2 at 10 WAT (Table 2). The number of thrips per plant was similar between the two entries at the other observation dates. One possible explanation for the difference in disease severity between these two entries, beyond thrips number, could be a difference in plant maturity. In 2013, plants of NMSU 07-32-2 were observed to mature at least two weeks earlier than plants of NMSU 12-335 (data not presented). This difference could mean that plants of the original and selected lines were not at the similar physiological stages when rated for disease severity and other traits. Plants closer to maturity tend to be more susceptible to stress and might exhibit more severe disease symptoms [12,16]. It is likely that at the second rating time, plants of NMSU 12-335 were actively growing and might have been more tolerant to stress and had not developed IYS as readily as maturing plants of NMSU 07-32-2. Another possibility could be that disease and associated stress could be causing plants to mature earlier, while plants of NMSU 12-335 that had fewer symptoms and seemed more tolerant to the disease could be maturing later. Higher expression of IYS symptoms on plants of NMSU 07-32-2 at a later rating date might be due to the reason that plants had already begun the senescence process and this could have affected the ratings. Several researchers have reported higher disease severity at later ratings [12,15,16] and difficulty in distinguishing between IYS symptoms and senescing plant tissues [15].

In 2013, plants of another selected breeding line, NMSU 12-236, exhibited fewer and less severe disease symptoms than its original population (NMSU 07-52-1) at 13 WAT (Table 1). Less severe symptoms were also observed at 19 WAT. In addition, plants of the selected breeding line, NMSU 12-796, also exhibited fewer and less severe disease symptoms than its original population (NMSU 07-55-1) at 13 WAT (Table 1). Less severe symptoms were also observed at 16 WAT. In 2013, plants of selected breeding lines NMSU 12-242 and 12-340 exhibited less severe symptoms when compared to plants of their original populations NMSU 07-53-1 and 07-56-2 at 16 WAT, respectively (Table 1). In each case mentioned, the differences in disease development arose even though there was no difference in the number of thrips per plant between the two entries at each observation time (Table 2).

As mentioned earlier, several selected breeding lines (NMSU 12-236, 12-243, 12-335, 12-337, and 12-796) in 2013 produced more plants without disease symptoms (lower disease incidence) than their respective original populations at the first rating date (13 WAT) (Table 1). For all but one of these selected lines, the number of thrips per plant at 10 WAT of these breeding lines was similar to their respective original populations (Table 2). By the second rating date, all asymptomatic plants in these breeding lines expressed disease symptoms. As mentioned earlier, a delay in symptom development and severity could be beneficial as it might generate a greater number of bulbs with less reduction in size and weight due to less leaf area affected by IYS disease.

In comparison to the susceptible check 'Rumba', most of the selected breeding lines exhibited less severe disease symptoms at most of the ratings. In 2013, plants of 'Rumba' exhibited disease severities of 1.6, 2.7 and 3.8 at 13, 16, and 19 WAT, respectively (Table 3). In 2014, plants of 'Rumba' exhibited less severe disease symptoms (0.6, 1.0 and 1.4) at the same rating times (Table 3). Most of the selected breeding lines evaluated in 2013 exhibited a lower disease severity than 'Rumba' at 13 WAT (Table 3). This lower disease severity likely resulted from the lower disease incidence observed for these breeding lines as compared to 'Rumba' (Table 3). At 13 WAT, some of the original populations also exhibited a lower disease severity than 'Rumba' (Table 3). While selection for reduced symptom expression was effective in the selected breeding lines, the original populations already possessed the means to express less severe symptoms when compared to 'Rumba'. For most of the selected breeding lines and original populations tested in 2013, their plants possessed a comparable number of thrips as plants of 'Rumba' (Table 2). The difference in symptom severity between 'Rumba' and the other entries was not likely because of thrips feeding. When thrips number per plant, disease severity, and disease incidence were less in 2014 as compared to 2013, there were fewer differences between plants of selected breeding lines and plants of 'Rumba' with respect to disease severity (Table 3). In 2014, plants of the selected breeding lines and the original populations possessed fewer thrips at all dates than plants of 'Rumba' (Table 2). In both years, plants of NMSU 12-239 exhibited less severe disease symptoms than plants of 'Rumba' at all three rating dates (Table 3). This consistency within a year and over years suggests that this selected breeding line should be evaluated further for its potential to produce plants with fewer disease symptoms when grown under high disease pressure. When compared to other commercial cultivars, plants of NMSU 12-243, 12-239, and 12-335 had less IYS and thrips damage and fewer thrips than plants of three commercial cultivars, Barbaro, Joaquin, and Vaquero when all were tested at the Malheur, Oregon, USA, experimental station in 2015 [28].

The above results showed progress in reducing IYS symptom expression after two cycles of selection. Further progress towards a reduction or delay in IYS symptom expression with additional cycles of selection may be possible as the selection method appears to be effective. While the presence of onion thrips is essential for the spread of *Iris yellow spot virus*, a large number of thrips per plant may not be predictive for the future severity and occurrence of Iris yellow spot symptoms. Other factors besides thrips number per plant may influence disease severity and incidence. The success rate can be improved if effective disease screening methods such as use of mechanical inoculations [16] can be used for reducing IYS symptom expression. Performing selection on the selected breeding lines that showed promising results and then evaluating the further generations with the original breeding lines can be a future study project. Comparing these and subsequent selections with the original populations and checks at different locations or with more replications in each year will likely confirm the progress made for enhanced resistance. Furthermore, breeding lines that exhibit a delay in IYS disease development in different locations and years can be advanced as resistant lines and might be used to make new crosses with other breeding lines.

Table 3. Mean values and comparisons of selected lines with susceptible check 'Rumba' for Iris yellow spot (IYS) severity rating and incidence measured several times throughout the 2013 and 2014 growing seasons at the Leyendecker Plant Science Research Center in Las Cruces, New Mexico, USA.

	IYS Disease				
	severity rating [y]			incidence (%) [x]	
	Time after transplanting (weeks)				
Entry [z]	13	16	19	13	16
	2013				
NMSU 12-236	0.4 *	1.6 *	2.2 *	43.3	100.0
NMSU 12-238	0.7 *	1.5	2.3	70.0	100.0
NMSU 12-239	0.5 *	1.1 *	2.2 *	60.0	100.0
NMSU 12-242	0.7 *	1.2 *	2.6	63.3	100.0
NMSU 12-243	0.5 *	1.1 *	1.6 *	53.3 *	100.0
NMSU 12-335	0.3 *	1.4 *	2.7	33.3 *	100.0
NMSU 12-337	0.4 *	1.2 *	1.8 *	40.0 *	100.0
NMSU 12-340	1.1 *	2.3 *	3.6	90.0	100.0
NMSU 12-342	0.9 *	2.1 *	3.6	83.3	100.0
NMSU 12-796	0.7 *	1.9 *	3.3	63.3 *	100.0
NMSU 07-32-2	1.2	2.3 *	3.6	90.0	100.0
NMSU 07-52-1	1.1 *	1.9	2.8	90.0	100.0
NMSU 07-53-1	0.8 *	1.8	2.9	76.7	100.0
NMSU 07-54-1	0.7 *	1.9	2.7	85.0	100.0
NMSU 07-55-1	1.1 *	2.4	3.2	93.3	100.0
NMSU 07-56-2	1.3	2.6	3.7	93.3	100.0
Rumba	1.6	2.7	3.8	90.0	-
	2014				
NMSU 12-238	0.5	0.9	1.3 *	50.0	86.7
NMSU 12-239	0.1 *	0.5 *	1.3 *	13.3	53.3 *
NMSU 12-243	0.3	0.4 *	1.1 *	33.3	40.0 *
NMSU 12-342	0.7	1.0	2.0	70.0	100.0
NMSU 12-337	0.1 *	0.8	1.4	10.0	76.7
NMSU 07-53-1	0.2 *	0.9	1.1 *	20.0	93.3
NMSU 07-54-1	0.0 *	0.8	1.2 *	3.3	80.0
NMSU 07-56-2	0.3	1.0	1.4	30.0	96.7
Rumba	0.6	1.0	1.8	56.7	100.0

[z] Entries were tested in a randomized complete block design with three replications. 'Rumba' was a susceptible check in the study. Entry means with "*" as superscipt indicate that entries were significantly different from the susceptible check, 'Rumba', at $p \leq 0.05$. [y] Ten plants per plot were rated individually for IYS severity on a scale of 0 to 4, where 0 = no symptoms, 1 = 1 to 2 small lesions per leaf, 2 => 2 medium-sized lesions per leaf, 3 = lesions coalescing on more than 25% of the leaf, and 4 = more than 50% leaf death. [x] Percentage of plants showing IYS disease symptoms were calculated based on 10 plants per plot.

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