

Evaluation of resistance of USDA spinach germplasm to *Stemphylium vesicarium*

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Introduction

Leaf spot diseases of spinach, caused by *Stemphylium vesicarium* and *S. beticola* can reduce yield and quality, and be a major constraint in spinach production in the U.S. (du Toit and Derie 2001; du Toit and Hernandez-Perez 2005; Liu et al. 2020). Screening spinach germplasm to identify resistant material will be a critical component for the effective management of this disease.

The objective of this research was to evaluate USDA spinach germplasm and commercial cultivars to leaf spot disease caused by *S. vesicarium*.

Materials and Methods

Planting: Eight spinach seeds (total 317 USDA germplasm including lines and commercial cultivars) were planted in each pot (diameter: 10 cm, height: 10 cm) containing potting mix-LC1 (Sungro Horticulture Distribution Inc. Agawam, MA). Plant stands were thinned to three per pot, and three replicate pots per treatment were used (Liu et al. 2020).

Pathogen inoculation: The plants (35-days-old) were inoculated with a spore suspension (1 x 10⁵ spores/ml) (18-days-old cultures on PDA) of the isolate of *S. vesicarium* (Sb-1-St001) (Fig. 1). A non-inoculated control (Viroflay) was sprayed with distilled water and subjected to the same conditions. Tween (0.01%) was added to the conidial suspension and the water control. Plants were incubated in a mist chamber for 48 h at 20 to 22°C, and then moved to greenhouse (20 to 30°C) to observe disease development. The disease severity (DS) was recorded 7 to 16 days after inoculation (Liu et al. 2020). Disease symptoms were slightly different on leaves inoculated at different plant ages (Fig. 2)

Disease rating: The disease severity (DS) was scored on a disease scale of 0 to 4 as follows: 0 = no symptoms on leaves, 1 = 1 – 25% leaf showing symptoms of disease, 2 = 26 – 50%, 3 = 51 – 75%, and 4 = 76 – 100% leaf infected (Fig. 3). The mean disease rating was recorded 16 days after inoculation (Tables 1, 2 and 3).

Experimental design and statistical analysis: The scoring of the DS for each plant was converted to a percentage value (midpoints, whereby 0 = 0%, 1 = 12.5%, 2 = 25%, 3 = 62.5%, and 4 = 87.5%) (Villarrol-Zeballos et al. 2012). All analyses were performed using Statistical Analysis Systems software (PC-SAS 8.0; SAS Institute, Cary, NC).

Results and Discussion

For the screening of the USDA lines, forty percent of the USDA lines (118/294) were highly susceptible (DS = 4.0) to *S. vesicarium*. Thirty two percent of the USDA lines (95/294) were susceptible (DS ≤ 3.0 to 4.0) to *S. vesicarium*. Sixteen percent of the USDA lines (48/294) were moderately resistant (DS ≤ 2.0 to 3.0) to *S. vesicarium*. (Table 1)

Eight percent of the USDA lines (25/294) were moderately resistant (DS ≤ 1.0 to 2.0) to *S. vesicarium*, which included Palak (Sp114), Cornell ID #40, Yuhparo, Shami, Harlan 4403, Giant d'etc., Cornell ID #163, SV3580VC, 08-275, Prilepski, Palak (Sp124), Palak (Sp065), Cornell ID #215, Palak (Sp122), Universal, Responder, CGN 9503, Palak (Sp125), Victoria, 500, Cornell ID #82, Kora, Olbrzym zimowy, and Szekesfehervari (Table 1)

Four percent of the USDA lines (13/294) were highly resistant (DS ≤ 0 to 1.0) to *S. vesicarium*, which included Nostruosa wireflay, Silverwhale, 08-03-316-1(Fay), 08-03-316-1(Fay), 454, 499, 03-316_Old-7, 449, 497, New Asia, Supahku, I19A0286, and Cornell ID #72 (Table 1).

For the screening of the commercial cultivars, all cultivars showed some level of infection from Sb-1-St001 (*S. vesicarium*) (Table 3). Isolate Sb-1-St001 caused significant leaf damage with

Table 1. Resistance screen to *Stemphylium vesicarium* (Sb-1-St001) on USDA germplasm^a

Line name	Mean severity ^b	Standard error	t grouping ^c
Viroflay	4.0	0.2	a
El Prado	4.0	0.2	a
Platypus	4.0	0.2	ba
CGN 9627	4.0	0.2	a
CGN 9499	4.0	0.2	a
CGN 9615	4.0	0.2	a
CGN 9503	4.0	0.2	a
CGN 9639	4.0	0.2	a
CGN 9622	4.0	0.2	a
CGN 9619	4.0	0.2	a
08-275	1.6	0.2	vut
Prilepski	1.6	0.2	vut
Palak	1.6	0.2	vut
Palak	1.6	0.2	vut
Cornell ID #215	1.6	0.2	vut
Palak	1.3	0.2	vuuv
Universal	1.3	0.2	vuuv
Responder	1.3	0.2	vuuv
CGN 9503	1.2	0.2	vxw
Palak	1.2	0.2	vxw
Victoria	1.1	0.2	txw
500	1.1	0.2	txw
Cornell ID #82	1.1	0.2	txw
Kora	1.0	0.2	yzxw
Olbrzym zimowy	1.0	0.2	yzxw
Szekesfehervari	1.0	0.2	yzxw
Nostruosa wireflay	0.9	0.2	yzxa
Sliverwhale	0.9	0.2	yzxa
08-03-316-1(Fay)	0.9	0.2	yzxa
08-03-316-1(Fay)	0.9	0.2	yzxa
454	0.9	0.2	yzxa
499	0.8	0.2	yzba
03-316_Old-7	0.7	0.2	czba
449	0.7	0.2	czba
497	0.7	0.2	czba
New Asia	0.7	0.2	czba
Supahku	0.6	0.2	cba
I19A0286	0.6	0.2	cba
Cornell ID #72	0.3	0.2	cd
Viroflay	0	0	d

^a: Disease scale: 0 = no symptoms on leaves, 1 = 1 – 25%, 2 = 26 – 50%, 3 = 51 – 75%, and 4 = 76 – 100% leaf infected. ^b: Final disease severity for leaf spot at 16 days after inoculation. ^c: Different letters indicate disease severities were significantly different at *P* < 0.05 based on Fisher's protected least significant difference (LSD). LSD is 0.44.



Fig. 1. Conidia morphology of *S. vesicarium* (Sb-1-St001).

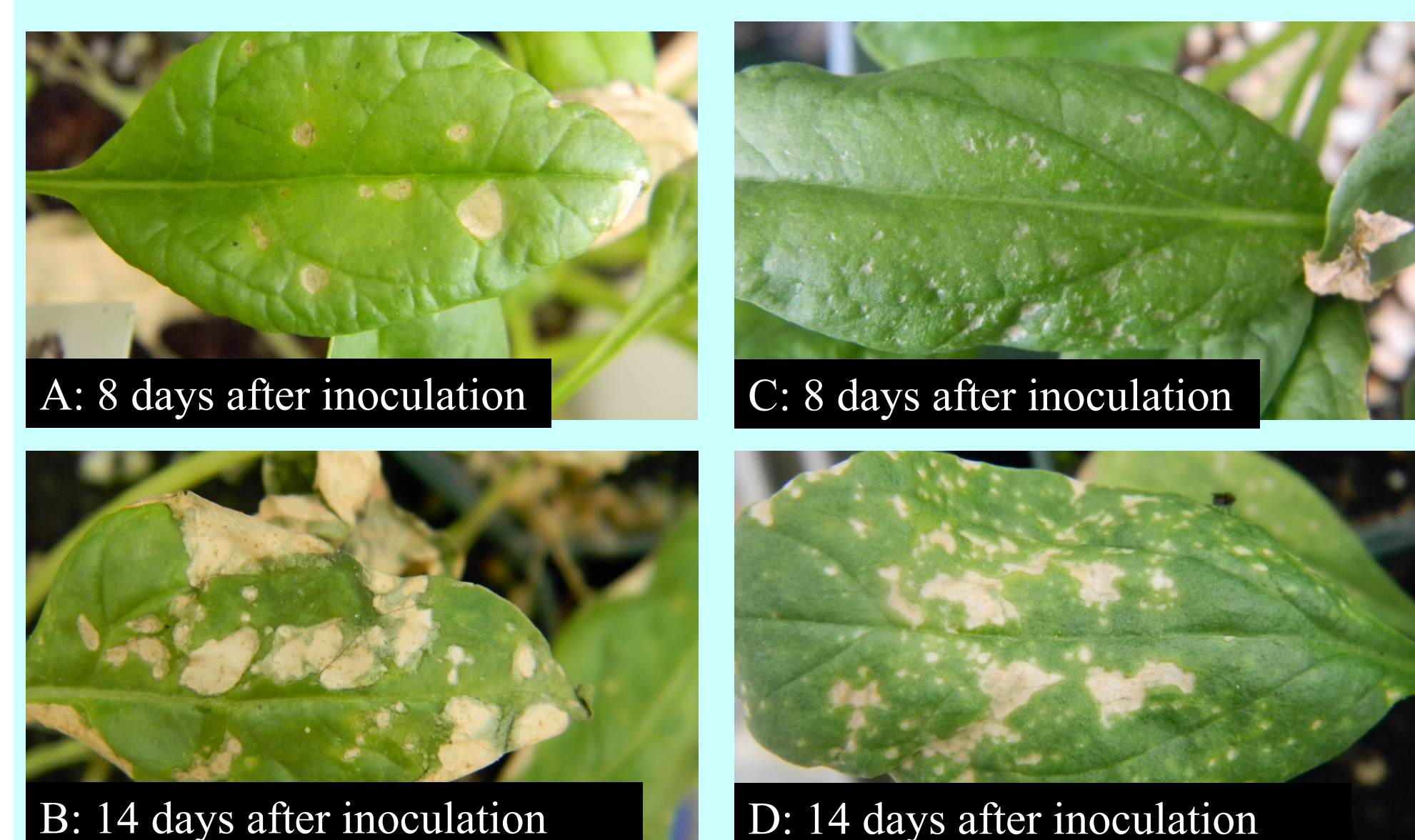


Fig. 2. Symptoms of leaf spots on the leaves of 35-day-old (A and B) and 45-day-old (C and D) plants, Disease developed much quicker and lesions expanded bigger on the leaves of 30-day-old than the ones of 45-day-old.

Table 2. Resistance screen to *Stemphylium vesicarium* (Sb-1-St001) on commercial spinach cultivars^a

Commercial cultivars	Mean severity ^b	Standard error	t grouping ^c
Viroflay	4.0	0.2	a
El Prado	4.0	0.2	a
Regor	4.0	0.2	a
PV 1477	4.0	0.2	a
Alcor	4.0	0.2	a
Volans	4.0	0.2	a
Finwhale	4.0	0.2	a
Platypus	4.0	0.2	ba
Resistoflay	3.8	0.2	bac
PV1512	3.6	0.2	bdec
Shelby	3.3	0.2	fddeg
PV1519	3.2	0.2	fheg
Lion	2.8	0.2	jiik
PV1446	2.2	0.2	mpqo
PV 1449	2.0	0.2	rspq
Califlay	2.0	0.2	rspq
PV 1452	1.2	0.2	vxw
PV 1445	1.1	0.2	txw
Whale	1.1	0.2	txw
Campania	1.0	0.2	yzxw
Lazio	0.7	0.2	czba
Tasman	0.4	0.2	cb
Viroflay water control	0	0	d

^a: Disease scale: 0 = no symptoms on leaves, 1 = 1 – 25%, 2 = 26 – 50%, 3 = 51 – 75%, and 4 = 76 – 100% leaf infected. ^b: Final disease severity for leaf spot at 16 days after inoculation. ^c: Different letters indicate the DS were significantly different at *P* < 0.05 based on Fisher's protected least significant difference (LSD). LSD is 0.44.

Table 3. Resistance screen to *Stemphylium vesicarium* (Sb-1-St001) on the selected USDA lines and commercial cultivars from Table 1^a

Cultivars and lines	Mean severity ^b	Standard error	t grouping ^c
Com. variety Viroflay	3.1	0.1	a
Com. variety Platypus	3.0	0.1	a
Com. variety El Prado	2.8	0.1	b
USDA lines CGN 9623	2.7	0.1	b
USDA lines Harlan 390	2.2	0.1	c
USDA lines Cornell ID #65	2.0	0.1	d
USDA lines 134 X 129	1.7	0.1	edf
USDA lines SPI 111/80	1.7	0.1	edf
Com. variety Tasman	1.2	0.1	gf
USDA lines PV 1477	1.1	0.1	hgf
USDA lines Universal	1.1	0.1	hgf
USDA lines 08-03-316-1(Fay)	0.9	0.1	hgi
Com. variety PV 1445	0.7	0.1	hjgi
USDA lines New Asia	0.7	0.1	hjgi
USDA lines Sliverwhale	0.6	0.1	jki
USDA lines I19A0286	0.6	0.1	jki
USDA lines 08-03-316-1(Fay)	0.3	0.1	jki
USDA lines 449	0.3	0.1	jki
Com. variety Lazio	0.2	0.1	jki
Com. variety Viroflay water control	0	0	

^a: Disease scale: 0 = no symptoms on leaves, 1 = 1 – 25%, 2 = 26 – 50%, 3 = 51 – 75%, and 4 = 76 – 100% leaf infected. ^b: Final disease severity for leaf spot at 16 days after inoculation. ^c: Different letters indicate disease severities were significantly different at *P* < 0.05 based on Fisher's protected least significant difference (LSD). LSD is 0.40.

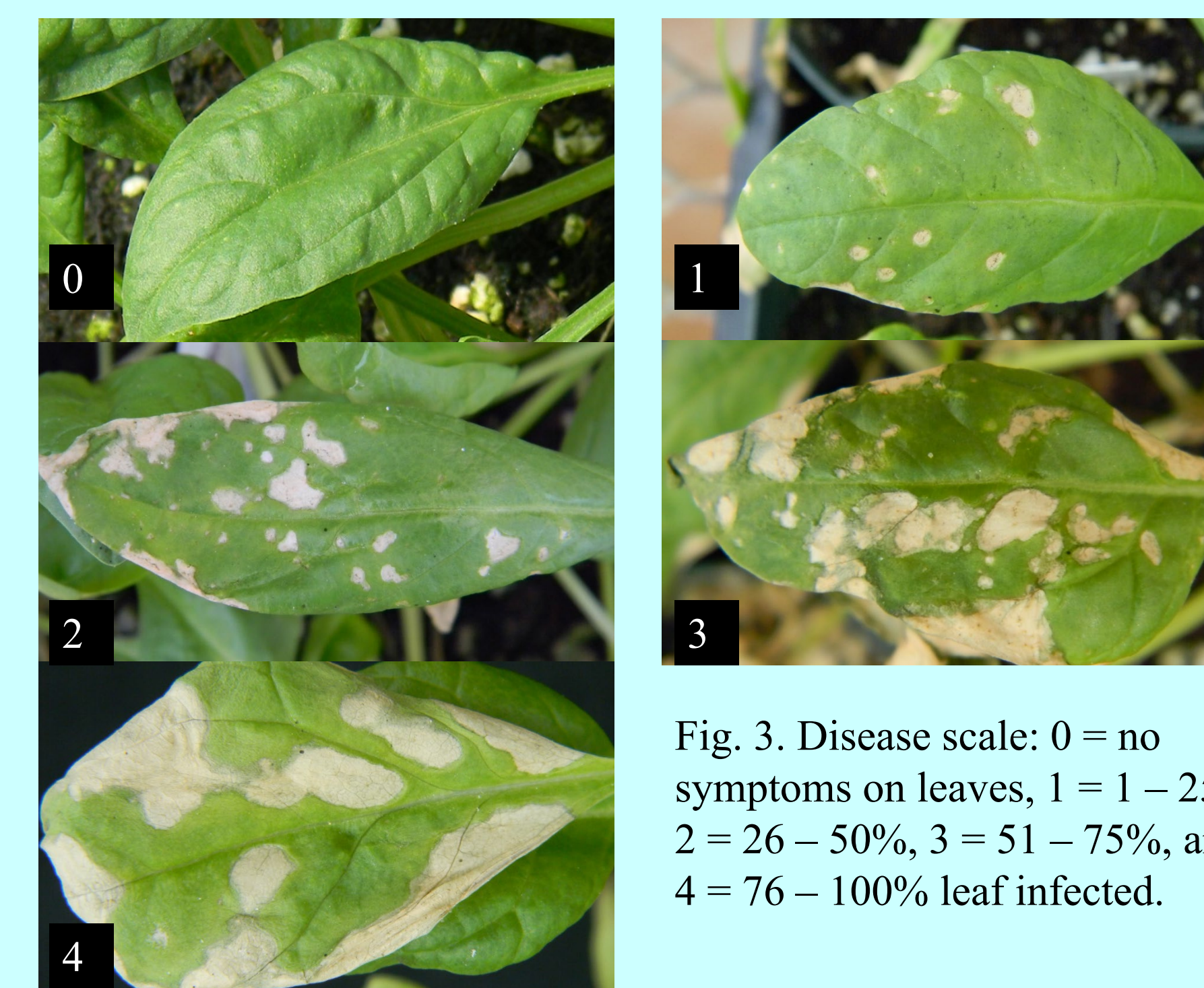


Fig. 3. Disease scale: 0 = no symptoms on leaves, 1 = 1 – 25%, 2 = 26 – 50%, 3 = 51 – 75%, and 4 = 76 – 100% leaf infected.

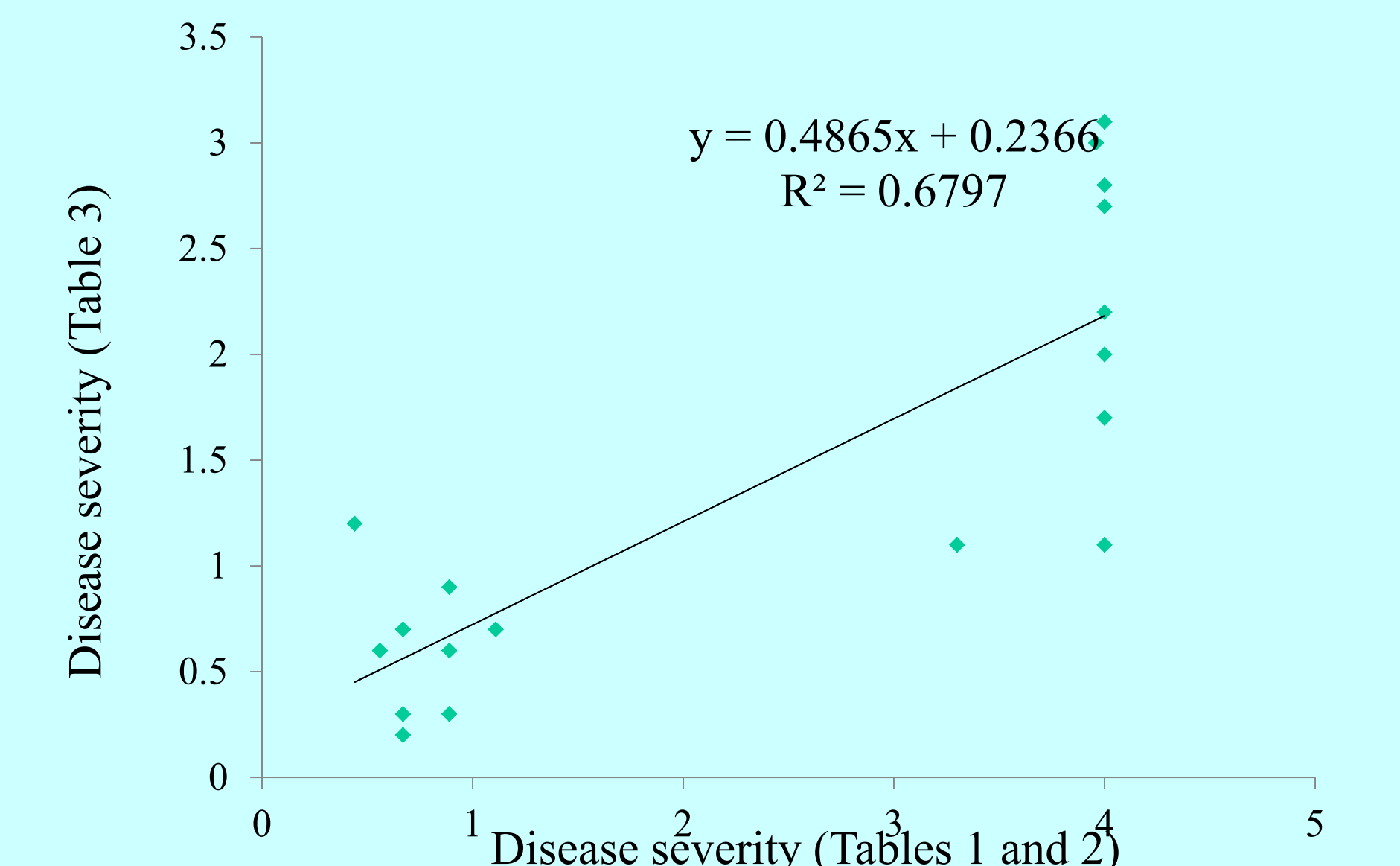


Fig. 4. Correlation and regression analysis between the DS of the test (Tables 1 and 2) and test (Table 3). Correlation coefficient ($R^2 = 0.6797$) indicates that the DS from both tests were significantly correlated.

the DS of 4.0 (76 to 100% lesion coverage) on the cultivars Viroflay, El Prado, Regor, PV1477, Alcor, Volans, Finwhale, and Platypus, and with the DS of 3.0 (51 to 75% lesion coverage) on the cultivars including Resistoflay, PV1512, Shelby, and PV1519. The isolate caused medium-level leaf damage with the DS of 2.0 (26 to 50% lesion coverage) on the cultivars Lion, PV1446, PV 1449, and Califlay. The isolate caused much less disease (DS = 1 to 25% lesion coverage) on the cultivars PV 1452, Whale, and Campania. (Table 3). The cultivars PV1452, Whale, and Campania exhibited the highest relative level of resistance to *S. vesicarium* (Table 2).

A list of USDA lines and commercial cultivars (Table 3) were selected from the large-scale screening (Tables 1 and 2), to determine the consistency in the relative disease reactions to *S. vesicarium*. All the resistant USDA lines and commercial cultivars in Table 3 followed the same categories of susceptible or resistance in Tables 1 and 2.

Correlation and regression analysis between the DS of the screening of USDA lines and commercial cultivars (Tables 1 and 2) and the selected ones for repeating (Table 3) showed that the DS from both tests were significantly correlated ($R^2 = 0.6797$) (Fig. 4).

Conclusion

Phenotypic evaluation of the USDA germplasm and commercial cultivars identified a number of lines and commercial cultivars that were resistant to *Stemphylium* leaf spot, and this information could be used to accelerate disease resistance breeding.

References

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