Evaluation of Seed Treatments for Reducing *Alternaria porri* and *Stemphylium vesicarium* on Onion Seed

THERESA A. S. AVELING and H. G. SNYMAN, Margaretha Mes Institute for Seed Research, University of Pretoria, Pretoria 0002; and S. P. NAUDE, Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria 0001, Republic of South Africa

ABSTRACT

Aveling, Theresa A. S., Snyman, H. G., and Naude, S. P. 1993. Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. Plant Dis. 77:1009-1011.

During surveys in the Cape province of South Africa, *Alternaria porri* and *Stemphylium vesicarium* were found to be destructive seedborne pathogens of onion (*Allium cepa* L.). Six fungicides were evaluated for their efficacy in reducing pathogens both on seed and in culture. These fungicides included anilazine, benomyl, a carbendazim/flusilazole mixture, procymidone, tebuconazole, and thiram. An untreated control, hot water soak (50 C for 20 min), and a sodium hypochlorite treatment were also included for comparison. Treated seeds were rated for germination by the blotter method and by emergence and seedling growth in seedling trays in the glasshouse. None of the treatments eradicated *A. porri* and *S. vesicarium* from onion seeds. The hot water soak proved to be the best treatment for reducing these pathogens, although the percentages of germination and emergence of onion seeds were reduced compared to the control.

Purple or Alternaria blotch, caused by *Alternaria porri* (Ellis) Cif., is a common disease of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) occurring wherever these crops are grown in South Africa (1,6). The first symptoms on onion leaves appear as numerous, white, irregular spots, which under favorable conditions gradually enlarge and become dark purple and brown (8). When seed stalks are girdled by the pathogen, they may break and fall over before the seed matures (2). Recent surveys in the Cape province of South Africa have shown that *Stemphylium vesicarium* (Wallr.) E. Simmons, in conjunction with *A. porri,* is also a destructive foliar and seed stalk pathogen of onion under warm, moist conditions (T. A. S. Aveling, H. G. Snyman, and S. P. Naude, *unpublished*). Because both *A. porri* and *S. vesicarium* are seedborne in onion (7), a seed treatment that could control or eradicate both pathogens simultaneously could be highly advantageous and would eliminate a potential source of inoculum in the field.

Accepted for publication 26 May 1993.

Currently, thiram is used as a standard treatment in South Africa for reducing fungal seedborne pathogens of vegetables (9). Maude (4) found that thiram eliminated infection of carrot seeds by *Alternaria dauci* (Kühn) Groves & Skolko and *Stemphylium radicinum* (Meier, Drechs., & E.D. Eddy) Neergaard. However, Maude et al (5) found that although a thiram soak completely eradicted 16 seedborne fungal pathogens of vegetable, cereal, and flower seeds, it did not completely eradicate *Alternaria brassicicola* (Schwein.) Wiltshire from brassica seeds. They also found that the thiram treatment adversely affected the germination of some vegetable seeds (5).

The objectives of this study were to evaluate the effectiveness of several other fungicides and hot water for eradicating *A. porri* and *S. vesicarium* from naturally infected onion seeds and to determine the effects of these treatments on germination, emergence, and seedling growth.

MATERIALS AND METHODS

Fungicide treatments. The following fungicides were used in in vitro and in vivo experiments (the trade name and recommended dosage per kilogram of seed are given in parentheses): thiram (Thiulin, 0.9 g a.i./kg); benomyl (Benlate, 1 g a.i./kg); anilazine (Dyrene, 1.6

Table 1. Diameter of colonies of *Alternaria porri* and *Stemphylium vesicarium* grown on potato-dextrose agar amended with various fungicides and sodium hypochlorite

Treatment	Rate (g a.i./L media)	Diameter of colonies (mm)[z]	
		A. porri	*S. vesicarium*
Control	...	21.62 a	37.52 a
Tebuconazole	0.05	6.22 f	2.53 i
	0.025	6.90 f	4.15 h
Carbendazim/ flusilazole mixture	0.19/0.38	0.00 i	0.00 j
	0.095/0.19	0.00 i	0.00 j
Procymidone	0.5	1.30 h	0.00 j
	0.25	1.53 h	0.00 j
Thiram	0.9	21.13 a	23.75 b
	1.35	18.60 b	20.67 d
Anilazine	1.6	4.23 g	22.45 c
Benomyl	1.0	13.92 c	10.58 f
	1.5	12.30 d	8.90 g
NaOCl	1%	8.98 e	17.70 e
	1.5%	7.33 f	11.32 f

[z]Each value is a mean of six plates measured after 6 days of growth. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Percent germination of treated onion seed in the laboratory and percent emergence and seedling growth of onion in the glasshouse after various seed treatments

Treatment	Rate (g a.i./kg)	Germination (%)[y]	Emergence (%)[z]	Shoot length (mm)[z]	Root length (mm)[z]
Control	...	75.5 a	75.0 b	186.6 bcd	47.79 a
Hot water	...	70.8 b	69.0 cd	181.5 bc	50.16 ab
NaOCl	1%	56.5 efg	65.0 def	176.9 abc	46.01 a
	1.5%	44.5 i	NT	NT	NT
Tebuconazole	0.05	58.5 de	65.0 def	164.9 a	48.08 a
	0.025	74.0 ab	80.0 a	197.3 d	56.56 c
Thiram	0.9	61.3 cd	66.0 def	181.0 bc	53.97 bc
	1.35	51.3 h	NT	NT	NT
Anilazine	1.6	61.5 cd	67.0 de	166.6 a	53.51 bc
	2.4	57.3 ef	NT	NT	NT
Benomyl	1.0	56.5 efg	68.0 cde	172.8 ab	46.45 a
	1.5	53.5 gh	NT	NT	NT
Carbendazim/ flusilazole mixture	0.19/0.38	54.5 fgh	64.0 ef	163.2 a	49.88 ab
	0.095/0.19	62.5 c	68.0 cde	188.6 cd	56.49 c
Procymidone	0.5	56.3 efg	62.0 f	163.2 a	49.29 ab
	0.25	63.3 c	72.0 bc	172.9 ab	56.64 c

[y]Each value is a mean of four replicates of 400 seeds.
[z]Each value is a mean of four replicates of 50 seedlings. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test. NT = not tested because results from in vivo and germination tests indicated that these treatments were unsuitable for onion seeds.

Table 3. Percent infection by *Alternaria porri* and *Stemphylium vesicarium* of treated and untreated onion seed and of seedlings grown from this seed

Treatment	Rate (g a.i./kg)	Infection of seed (%)[y]		Infection of seedlings (%)[z]	
		A. porri	*S. vesicarium*	*A. porri*	*S. vesicarium*
Control	...	6.5 cde	32.0 d	7.0 b	27.5 c
Tebuconazole	0.05	3.5 ab	23.0 bc	2.0 a	15.5 b
	0.025	4.0 abc	23.5 bcd	NT	NT
Carbendazim/ flusilazole mixture	0.19/0.38	3.5 ab	22.0 bc	1.5 a	14.5 b
	0.095/0.19	4.0 abc	25.5 bcd	NT	NT
Procymidone	0.5	4.0 abc	23.5 bcd	2.5 a	21.5 bc
	0.25	7.0 de	30.0 cd	NT	NT
Thiram	1.35	7.0 de	21.0 b	NT	NT
Anilazine	2.4	8.0 e	19.0 b	6.0 b	15.5 b
Benomyl	1.5	5.0 bcd	25.0 bcd	NT	NT
NaOCl	1.5%	5.0 bcd	21.0 b	NT	NT
Hot water	...	2.0 a	1.0 a	1.0 a	0.5 a

[y]Each value is a mean of four replicates of 100 seeds.
[z]Each value is a mean percentage of four replicates of 50 seeds. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test. NT = not tested because results from in vivo and in vitro experiments indicated that these treatments were unsuitable for onion seeds.

g a.i./kg); a carbendazim/flusilazole mixture (Punch C, 0.19/0.38 g a.i./kg); tebuconazole (Raxil, 0.05 g a.i./kg); and procymidone (Sumisclex, 0.5 g a.i./kg). Because no fungicides other than thiram are registered in South Africa for the treatment of onion seed, we relied on the expertise of the chemical companies (Agrihold, South Africa, and Bayer, South Africa [Pty] Ltd) to supply the fungicides and the recommended dosage. The above rates were also used as standard rates per liter of medium in the in vitro experiment.

In vitro experiment. *A. porri* and *S. vesicarium* were isolated from leaves of diseased onion plants, and a virulent isolate of each was maintained on potato-dextrose agar (PDA) at 20 C in the dark. These isolates of *A. porri* (PREM 50715) and *S. vesicarium* (PREM 50717) were deposited with the National Collection of Fungi, South Africa.

Sodium hypochlorite (1%) and each test fungicide, at the standard rate, was incorporated into PDA. Disks (3 mm in diameter) from actively growing cultures of *A. porri* and *S. vesicarium* were transferred aseptically to amended plates and to control plates. Six replicate plates of each treatment for each pathogen were incubated at 20 C in the dark. The diameter of the colony was measured after 3, 6, and 10 days of incubation. Each assay was repeated once. Depending on the results of these experiments with the recommended dosage (1×), 0.5 or 1.5× the recommended dosage was also tested.

In vivo experiment. Four replicates of 100 onion seeds from two seed samples of the cultivar Caledon Globe were plated on PDA and incubated at 20 C in a 12-hr light/dark regime. Results of these experiments indicated that one seed sample was naturally infected with *A. porri* and *S. vesicarium,* while the other was not infected with either pathogen. These two seed samples were used in in vivo experiments.

Based on the results obtained from the in vitro experiments, the treatments were applied to the two seed samples at 1, 0.5, and 1.5× the recommended dosage. Each of the chemicals was suspended in 1 ml of sterile distilled water and applied as a slurry to a 20-g seed sample. The control was treated with 1 ml of sterile distilled water. The treated seeds were allowed to air-dry overnight. Twenty grams of seed was also treated with a hot water soak (50 C for 20 min).

Seed germination assays. *Laboratory tests.* The effect of the various treatments on the percentage of germination was determined according to the rules of the International Seed Testing Association (ISTA) (3). Four replicates of 100 uninfected seeds from each treatment were plated on moist blotter paper in glass petri dishes and incubated at 20 C in a 12-hr light/dark regime. The experiment was repeated once. The efficacy of the

various treatments in reducing seed infection by *A. porri* and *S. vesicarium* was also tested. Four replicates of 100 naturally infected seeds from each treatment were plated onto PDA. The untreated seeds yielded the level of infection of the seed. The petri dishes were incubated at 20 C in a 12-hr-dark/12-hr-ultraviolet light regime. Three and six days later, the numbers of *A. porri* and *S. vesicarium* colonies growing from the seeds were recorded. The experiment was repeated once.

Glasshouse tests. Four replicates of 50 uninfected seeds of each treatment (1 and 0.5× the recommended dosage) and four of 50 naturally infected seeds of each treatment (1 and/or 0.5 or 1.5× the recommended dosage) were planted in seedling trays containing a peat-based growing medium, maintained in a glasshouse (25/20 C day/night temperature), and watered daily. Four weeks after planting, the percentage of emergence and the shoot and root length of seedlings from uninfected seed were determined. The experiment was a randomized block design repeated once. Leaf samples from seedlings from treated, infected seed which showed symptoms of *A. porri* and/or *S. vesicarium* were surface sterilized with 1% NaOCl before plating on PDA or were placed directly onto moist blotter paper in glass petri dishes. The plates and petri dishes were incubated at 20 C in a 12-hr-dark/12-hr-ultraviolet light regime. Three and six days later, the tissues were examined for the presence of sporulation, and spores were identified as either *A. porri* or *S. vesicarium*.

RESULTS

In vitro experiment. All of the fungicide and sodium hypochlorite treatments significantly inhibited mycelial growth of *A. porri* in culture when compared to the control, with the exception of thiram at the standard rate (Table 1). Mycelial growth of *S. vesicarium* was significantly inhibited by all the fungicide and sodium hypochlorite treatments when compared to the control (Table 1). It is evident from the results presented in Table 1 that the three fungicides tebuconazole, the carbendazim/flusilazole mixture, and procymidone were most effective at inhibiting both pathogens in culture.

In vivo experiments. All treatments, with the exception of tebuconazole at half the recommended dosage, significantly reduced the percentage of germination of onion seed on blotter paper when compared to the control (Table 2).

With the exception of tebuconazole and procymidone at half the recommended dosage, all treatments significantly reduced the percentage of emergence in glasshouse experiments when compared to the uninfected control (Table 2). Four treatments (tebuconazole, anilazine, the carbendazim/flusilazole mixture, and procymidone, all at the recommended dosage) also significantly reduced shoot growth (Table 2). Half the recommended dosage of tebuconazole, the carbendazim/flusilazole mixture, and procymidone did not inhibit shoot growth. Five treatments stimulated root length significantly compared to the control. These included thiram and anilazine at the recommended dosage and tebuconazole, the carbendazim/flusilazole mixture, and procymidone at half the recommended dosage (Table 2). Only three treatments did not significantly reduce dry shoot and root mass compared to the control, i.e., thiram and anilazine at the recommended dosage and tebuconazole at half the recommended dosage (Table 2).

The percentage of onion seeds and seedlings infected with *A. porri* and *S. vesicarium* grown from treated and untreated naturally infected seed are presented in Table 3. Only three treatments significantly reduced the percentage of infection of onion seed by *A. porri* compared to the naturally infected control. These were the hot water treatment and the tebuconazole and carbendazim/flusilazole mixture treatments at the recommended dosage (Table 3). These three treatments and thiram and anilazine (both at 1.5× the recommended dosage) also significantly reduced percentage of infection by *S. vesicarium* (Table 3). Of all the treatments tested in the glasshouse, only anilazine failed to control *A. porri,* and only procymidone failed to control *S. vesicarium.* The hot water soak was most effective in reducing both pathogens simultaneously in the laboratory and the glasshouse (Table 3).

DISCUSSION

According to Neergaard (7), effective seed treatments must eliminate pathogens without being toxic to seeds. All the fungicides incorporated into PDA, except thiram at the standard rate, reduced the growth of both pathogens, with the most effective being tebuconazole, the carbendazim/flusilazole mixture, and procymidone. Only tebuconazole and the carbendazim/flusilazole mixture, at the recommended dosage, also effectively reduced the percentage of infection of onion seed and seedlings by both pathogens in laboratory and glasshouse experiments, while only tebuconazole at half the recommended dosage did not significantly reduce germination in laboratory experiments. In glasshouse experiments, however, neither tebuconazole nor procymidone at half the recommended dosage significantly reduced the percentage of emergence, although procymidone did have an adverse effect on seedling growth.

None of the seed treatments eradicated the two pathogens. The chemicals either are not fungitoxic to the pathogens or did not penetrate the seed tissues to kill internal mycelia. The hot water soak was the most effective treatment for reducing both pathogens in laboratory and glasshouse tests. However, this treatment significantly reduced the percentage of germination and emergence compared to the control. It is possible that by varying the temperature of the water and/or the submergence time, the percentage of germination and emergence might be maintained while reducing the two pathogens.

The efficacy of the seed treatments was not tested under field conditions.

ACKNOWLEDGMENTS

This research was supported by a grant from the Agricultural Research Council. The research is part of a Ph.D. thesis to be submitted by the first author to the University of Natal, Pietermaritzburg. The authors thank B. H. Boelema for proofreading the manuscript.

LITERATURE CITED

1. Aveling, Theresa A. S., and Naude, S. P. 1992. First report of *Alternaria porri* on garlic in South Africa. Plant Dis. 76:643.
2. Boelema, B. H., and Ehlers, J. L. 1967. Alternaria-blotch of onions. Farming S.A. 43:15.
3. International Seed Testing Association. 1985. International rules for seed testing, 1985. Seed Sci. Technol. 13:299-355.
4. Maude, R. B. 1966. Studies on the etiology of black rot *Stemphylium radicinum* (Meier, Dreschl. & Eddy) Neerg. and leaf blight, *Alternaria dauci* (Kuhn) Groves & Skolko, on carrot crops; and on fungicide control of their seed-borne infection phases. Ann. Appl. Bot. 57:87-93.
5. Maude, R. B., Vizor, A. S., and Shuring, C. G. 1969. The control of fungal seed-borne diseases by means of a thiram seed soak. Ann. Appl. Bot. 64:245-257.
6. Naude, S. P. 1988. Purple blotch of onions. S. Afr. Veg. Grow. April/May:19.
7. Neergaard, P. 1977. Seed Pathology. Macmillan Press, London.
8. Nolla, J. A. B. 1927. A new Alternaria disease of onions (*Allium cepa* L.). Phytopathology 17:115-132.
9. Vermeulen, J. B., Sweet, S., Krause, M., Hollings, N., and Nel, A. 1990. A Guide to the Use of Pesticides and Fungicides in the Republic of South Africa. 34th ed. Government Printer, Pretoria.