SEEDLING FAILURE IN ATRIPLEX (CHENOPODIACEAE) DUE TO "DAMPING-OFF"[1]

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Abstract: Repeated failures in attempts to establish a selection of Atriplex in a non-mined site near the Navajo Mine in New Mexico proved to be due to "damping-off" (black root) of the seedlings, caused by Pythiaceae fungi. The contaminated soil contained more than 1,000 propagules of the fungus per g of soil; stockpiled soil in another area contained an average of 20 propagules per g of soil. Coating the Atriplex seed with fungicide prior to sowing increased seedling establishment more than 100-fold. Reduced vigor and loss of plants subsequent to seedling-establishment may also be caused by these fungi.

Additional Key Words: Mine Reclamation; Pythium; Fungicides.

Introduction

In October, 1990, a ten-acre (4-ha) plot near the Yazzie pit, at the BHP Navajo Mine, San Juan Co., New Mexico, was seeded to a selection of Atriplex derived from the hybrid Atriplex canescens (Pursh.) Nutt. x A. obovata Moq. (ATCA x ATOB). Surprisingly, it was a complete failure; not one viable seedling could be found by August, 1991.

Earlier studies had indicated high performance of this selection viz:

1. Both parents had shown a high level of adaptability in study plots at the Navajo mine.
2. F_1 hybrids are vigorous and fertile.
3. Second, third, fourth and fifth generation segregants all showed high fertility and high adaptability.
4. Seed germination was high in the F_1 and subsequent generations.
5. Shelf-life of the seeds is high (at least 3 years without any signs of decrease in viability).
6. A vast array of successful segregant genotypes are conspicuous in all previous outplanting trials.
7. Volunteer seedlings have become established in older (3 years +) plantings.
8. Trial revegetation plots of this selection have all shown excellent performance. (2 ha at Doby, ramp 12, Navajo Mine; 4 ha at Watson, Navajo Mine; 2 ha at Pinto, Navajo Mine; 6 ha at Gravel Hill, San Juan Mine.

Failure at the 4-ha Yazzie plot was therefore completely unexpected. Unable to account for this failure, a decision was made to replant the entire plot with a fresh batch of seed of the same parentage. This was accomplished on 5 March, 1992. In this instance approximately 50 lbs (ca 23kg) of Atriplex canescens x A. obovata (ATCA x ATOB) seed, hand harvested from a plot at Doby 12, Navajo Mine, was sown by drilling. In this operation 3/4 of the drills were plugged off so as to provide about 1 m between drill rows. After the 4-ha plot was sown, about 15 lbs (ca 7kg) of the same seed-lot was sown in an area of approximately 1 ha outside the fenced area.

In October, 1992, only a few seedlings were found in the 4-ha plot. They occurred mostly in clusters of approximately 5-10 seedlings in drill rows, followed by long distances devoid of seedlings. No seedlings were found in the seeded area outside the fence.

In order to optimize favorable conditions for the Atriplex canescens x A. obovata seedlings, approximately 6" (150mm) of water was applied to the plot in October 1992. In April, 1993, not one seedling could be found in the plot; not even those that had been seen the previous fall.

Methods and Results

In an attempt to discover the cause of this repeated failure, soil samples from the 4-ha plot were taken to the

[1]Paper presented at the 1995 National Meeting of the American Society for Surface Mining and Reclamation, Gillette, Wyoming, June 5-8, 1995.

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Proceedings America Society of Mining and Reclamation, 1995 pp 757-761
DOI: 10.21000/JASMR95010757
https://doi.org/10.21000/JASMR95010757

laboratory and sown with seed of the same lot that had failed in the Yazzie plot. As a control, seed was also sown in a commercial soil-mix (Sunshine mix #1). In 10-12 days, seedlings emerged in both soils but most of those in the Yazzie soil died of "damping-off" whereas only a few seedlings in the commercial mix succumbed. This dramatic difference invited pursuit of a more elaborate study to find if "damping-off" could be the cause of the repeated failures at the Yazzie plot.

Consequently the following three investigations were initiated: 1) detection and identification of the organisms causing the observed "damping-off,"
2) monitoring of "damping-off" of ATCA x ATOB seedlings derived from seeds treated with a fungicide and grown in Yazzie-plot soil, compared to seedlings derived from untreated seeds grown in Yazzie-plot soil and in commercial soil mix,
3) evaluation of the use of fungicide to control "damping-off" in field conditions.

1. Study of the organisms responsible for "damping-off."

a. Methods

Soil from the Yazzie plot and soil from a 10-year old stockpile were collected in sterile containers. Samples of these soils were prepared as outlined by Nelson et al. (1990), then introduced into petri-dishes containing sterile media [cornmeal agar containing P_5ARP [pimaricin, ampicillin, rifampicin and pentachloronitrobenzene; (Jeffers and Martin, 1986)], selective for determining the presence of pythiaceous fungi such as Pythium that can cause damping-off disease.

b. Results

In media containing P_5ARP, to which pythiaceous fungi are known to be resistant, 1230 propagules of pythiaceous fungi per gram of soil were present in soil from the Yazzie plot, but only 20 propagules per gram were present in soil from the stockpile (Table 1).

Table 1. Number of propagules of pythiaceous fungi in soil from the Yazzie plot and in soil from a stockpile.

Soil source	No. of propagules per g of soil
Yazzie	1230
Stockpile	20

2. Study of the effect of fungicide treatments on damping-off of ATCA x ATOB seedlings.

a. Methods

A number of seeds of ATCA x ATOB were placed in a bag containing the fungicide Captan and shaken until all seeds were coated with the fungicide and then removed by screening. One hundred of these treated seeds were sown in each of four pots containing soil from the Yazzie 4-ha plot. Four other pots were each sown with 100 untreated seeds. As controls, 100 untreated seeds were sown in each of four pots containing a commercial soil mix (Sunshine #1).

Each pot received 200 cc of water daily and the number of live and dead seedlings was recorded daily.

b. Results

As shown in Tables 2,3, and 5, mortality of ATCA x ATOB seedlings, grown in Yazzie-plot soil, was more than three times greater for seedlings derived from untreated seeds, than for seedlings derived from seeds treated with fungicide (87% vs. 27.5%). Survival of seedlings derived from untreated seeds when grown in commercial soil mix was much greater than when grown in Yazzie-soil (61.6% vs. 13.0%) but not significantly different than for seedlings derived from treated seeds (61.6% vs. 72.5%). (Tables 2,3,4,5)

Table 2. Seed germination and seedling survival of Atriplex canescens x A. obovata in Yazzie soil.

Pot #	# of seeds sown	Total # of seedlings	# of dead seedlings	# of live seedlings	% survivors
1	100	55	55	0	0
2	100	51	36	15	29.4
3	100	35	35	0	0
4	100	59	48	11	18.6
Total	400	200	174	26	13.0

Table 3. Seed germination and seedling survival of Atriplex canescens x A. obovata grown in Yazzie soil from seeds coated with the fungicide Captan.

Pot #	# of seeds sown	Total # of seedlings	# of dead seedlings	# of live seedlings	% survivors
1	100	65	15	50	76.9
2	100	68	22	46	67.6
3	100	85	24	61	71.8
4	100	87	23	64	73.6
Total	400	305	84	221	72.5

Table 4. Seed germination and seedling survival of Atriplex canescens x A. obovata in commercial soil mix (Sunshine #1).

Pot #	# of seeds sown	Total # of seedlings	# of dead seedlings	# of live seedlings	% survivors
1	100	52	21	31	59.6
2	100	49	17	32	65.3
3	100	94	32	62	65.9
4	100	86	38	48	55.8
Total	400	281	108	173	61.6

Table 5. Summary of Atriplex canescens x A. obovata seedling mortality in seedlings derived from untreated seeds grown in Yazzie soil, from seeds coated with the fungicide Captan before seeding and from seeds grown in commercial soil mix. Values in each column with the same letter are not significantly different (P < .05).

Treatment	seeds sown	germinants	dead seedlings	live seedling	% seedling survivors
Untreated seeds in Yazzie soil	400	200^{A}	174^{A}	26^{B}	13.0^{B}
Treated seeds in Yazzie soil	400	305^{A}	84^{B}	221^{A}	72.5^{A}
Untreated seeds in commercial soil	400	281^{A}	128^{AB}	173^{A}	61.6^{A}

3. Studies of the effect of fungicide in controlling "damping-off" in field conditions.

a. Methods

In August, 1993, the entire 4-ha Yazzie plot was disced, the dead remains of old Salsola kali L. plants were removed and the plot was reseeded with fresh ATCA x ATOB seed. Approximately 40% of the 4-ha plot (1.6 ha) was sown with seed that had been coated with the fungicide Captan; 40% (1.6 ha) was sown with untreated seed; an area of about 0.4 ha received transplants of year-old containerized ATCA x ATOB plants; the remainder of the 4-ha plot (ca O.4 ha) received no further seeding.

In October, 1993, among the few ATCA x ATOB seedlings that were found in the plot seeded with treated seed, 16 were marked with red pin flags in order to monitor their over-winter survival.

b. Results

In March, 1994, numerous seedlings were found in the area that had been seeded with fungicide-coated seed; none were found in the area that had been seeded with untreated seed and none were found in the non-reseeded area. Of the 16 plants that had been marked with pin-flags in October, 1993, only 9 remained alive.

In order to obtain a more accurate appraisal of seedling establishment and survival in the seeded areas, 5 linear, 1 m x 30 m transects, were used to estimate seedling density in the two contrasting reseeded areas. As shown in Table 6, 199 seedlings (ca 1/33 per m^2) were counted in the transects in the area seeded with fungicide-coated seed; only 2 seedlings (0.01 per m^2) were found in the transects in the area sown with untreated seed. No seedlings were found in the 0.4 ha area that was not reseeded in 1993. Many of the ATCA x ATOB transplants had died but the cause of death appears to be from rabbits rather than fungi.

Table 6. Density of seedlings of Atriplex canescens x Atriplex obovata in plots sown with seeds coated with fungicide, compared to those sown with untreated seeds.

Seed Treatment	Number of Seedlings	Transect size	Seedlings per m^2
Coated with fungicide	199	5 ea, 1m x 30m = 150m	1.33
Untreated	2	5 ea, 1m x 30m = 150m	0.01

Discussion

Failure to establish a stand of Atriplex at the Yazzie plot is apparently the result of damaging effects of pathogenic fungi as evidenced by the following observations: (1) a high rate of "damping-off" of seedlings grown in the greenhouses at Brigham Young University, Provo, Utah in soil taken from the Yazzie plot (87%), compared to seedlings grown under the same conditions in a commercial soil mix (38.4%); (2) a great reduction in seedling loss to damping-off when seeds were coated with fungicide (CAPTAN) before sowing in Yazzie-plot soil, compared to seedling loss with untreated seeds in the same Yazzie-plot soil and (3) by the dramatic improvement (13,300%) in seedling establishment, in field conditions at the Yazzie plot, when seeds were coated with fungicide (CAPTAN) prior to seeding (1.33 seedlings per m^2), compared to seedling establishment in areas where untreated seeds were sown (0.01 seedlings per m^2).

Continued damaging effects of pathogenic fungi after germination and seedling establishment is suggested by the loss of established seedlings (7 of 16 marked seedlings) after their post-emergence period of high susceptibility to damping-off. Seedling loss in commercial soil-mix sown with untreated seed suggests that seed-borne fungal propagules may be responsible for some of the infestation.

The low number of propagules of Pythiaceous fungi in the top-soil stockpile (20 per g) compared to the much higher number in Yazzie-plot soil (1230 per g) may be due to the origin of the topsoil from an area low in pathogenic fungi responsible for damping-off, or, alternatively, the stockpile experience may have been damaging to causative fungal propagules.

The discovery of severe damage of pathogenic fungi to Atriplex seedlings presents some important queries, such as:

1. How widespread is damping-off in wildland ecosystems? In agriculture, it has been identified to be severe and costly in many important crops, including beets and sugar beets (Pound 1953), members of the same family to which Atriplex belongs (Chenopodiaceae).
2. Is there wisdom in routinely treating seeds with fungicide, prior to sowing, in order to guard against pathogenic damage?
3. Does stockpiling reduce the number of propagules of pathogenic fungi?
4. Are there differences in susceptibility between species of Atriplex and other susceptible genera?
5. Can resistant strains be developed?
6. Are other reported (and unreported) instances of failures in revegetation efforts, the result of similar pathogenic infestations?
7. Will the use of fungicides have detrimental effects on other desirable organisms including naturally occurring damping-off antagonists?
8. Is the conspicuous absence of specific species in "empty" islands in some of our rangelands due to similar pathogenic assaults?

Hopefully, answers to these and other attending questions will lead to a better understanding of natural ecosystems and reclaimed disturbed lands, and how to best manage them.

Acknowledgments

Funding for these studies was provided by BHP-Minerals and Brigham Young University.

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DEVELOPMENT OF GENETIC DIVERSITY IN ATRIPLEX[1]

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Abstract: Polyploid plant taxa are usually low in genetic diversity and, consequently, are of limited value in revegetation of disturbed lands. This deficiency can be remedied in polyploid selections of Atriplex (saltbush) by using products of interspecific hybrids derived from parents having the same chromosome number and by using seed mixes derived from several contrasting ecotypes of the same species, all with the same chromosome number.

Additional Key Words: Polyploids; Hybrids; Reclamation; Die-off.

Introduction

The most conspicuous requirement for continued success of plant taxa in nature is genetic diversity. Species that are rich in genetic diversity "ride-out" environmental fluctuations; those that are uniform, face elimination.

In our efforts to reclaim disturbed sites we must therefore never use "pure-bred" introductions. This is particularly true for reclamation of mine spoils because the spoils are not only new and different but they are rapidly changing. Consequently a taxon, (particularly a genetically uniform taxon), that may appear to be a successful performer today, could be a complete failure later on.

The hazard accompanying low genetic flexibility is often seen in the valleys of the Great Basin of western United States where genetically uniform polyploid species experience extensive, sudden, "die-off." Many of these western valleys were covered with water as recently as 13,000 y.a. (Benson et al., 1990) and as the waters receded, vast new unoccupied landscapes became exposed which, in many respects, although on a much larger scale, resembled unoccupied, new mine-spoils. Because the valleys in the Great Basin have no drainage outlets, the lakes disappeared solely by evaporation and therefore left behind highly saline soils in the valley-bottoms. Since such soils are usually accomodated best by plants in the family Chenopodiaceae, Chenopods now dominate vast acreages throughout much of the Great Basin. Sarcobatus Nees, (Greasewood), Ceratoides Gagnebin, (winter-fat), Grayia H & A, (hop-sage), Kochia Roth, (gray molly), and Atriplex L., (saltbush) are the principal genera represented. Each of these, except Atriplex, contains only one or two species and are present throughout the Great Basin as genetically uniform monocultures or near monocultures. In contrast, the genus Atriplex contains numerous species and varieties, and hybridization, introgression, and polyploidization are common. As a consequence, some Atriplex immigrants in the Great Basin are severely genetically uniform, others are genetically rich.

On occasion, entire populations of Atriplex and other Chenopods that are genetically uniform, collapse. A striking example of this occurred in a population of A. confertifolia (Torr. & Frem.) Wats. (shadscale) in Rush Valley, Tooele Co., Utah. In this valley, shadscale plants above the high water level of Lake Bonneville are all diploid (2x=18) and show an abundance of genetic variation. Some are early-flowering, some late, some have broad leaves, some narrow, some have an erect, robust habit, some have a small, depauperate habit. At lower

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[1]Paper presented at the 1995 National Meeting of the American Society for Surface Mining and Reclamation, Gillette, Wyoming, June 5-8, 1995.

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