INTEGRATION OF SOLID MATRIX PRIMING WITH CHEMICAL AND BIOLOGICAL SEED TREATMENTS TO IMPROVE OKRA SEED GERMINATION AND REDUCE PREEMERGENCE DAMPING-OFF BY *PYTHIUM ULTIMUM*

By

RANJIT MEREDDY

Bachelor of Science

Andhra Pradesh Agricultural University

Hyderabad, India

1991

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1995

INTEGRATION OF SOLID MATRIX PRIMING WITH CHEMICAL AND BIOLOGICAL SEED TREATMENTS TO IMPROVE OKRA SEED GERMINATION AND REDUCE PREEMERGENCE DAMPING-OFF BY *PYTHIUM ULTIMUM*

Thesis Approved:

Kenneth E Conway
Thesis Advisor

John P. Damicone

Alexander B. J. Vonon

Thomas C. Collier
Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express special appreciation to my major advisor, Dr. K. E. Conway. He has provided essential guidance and inspiration throughout my graduate program. I would also like to thank my committee members, Dr. A. B. Filonow and Dr. J. Damicone, for their valuable suggestions during the course of this work.

Many thanks go to Dr. Claypool and Luguang Wu for statistical help and advice. I would like to express my appreciation to the Oklahoma Center for the Advancement of Science and Technology for their financial support.

I would like to extend my heartfelt thanks to all my colleagues and professors in the Department of Plant Pathology at Oklahoma State University.

Finally, I would like to express my sincerest thanks to my family for their continued support. They have helped me throughout my Master's program. I couldn't have done it without your continued love and support.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Okra *(Abelmoschus esculentus* (L) Moench) is a vegetable crop of moderate economic importance and is grown in Texas, Georgia, Florida, California, Tennessee, Alabama and Oklahoma. The cultivated land area of Okra in Oklahoma is about 650 acres and has a value of $ 2 million dollars annually (Dr. J. Motes, *personal communication).* Poor germination, emergence, and seedling uniformity are common problems in the production of all okra cultivars (Anderson et al., 1960; Standifer et al., 1989; and Marsh, 1992,1993). The hard seed coat of okra and damping-off diseases are the main reasons for this problem (Grand, L.F. 1985 and Marsh, 1992,1993).

Several researchers (Anderson et al., 1960; Edmond and Drapala, 1960; Abdelfattah et al., 1972; Standifer et al., 1989 and Marsh, 1992,1993) have attempted to address both the physiological (hard seed coat) and pathological (damping-off) problems of okra with limited success. Although specific fungicides such as metalaxyl (Ridomil) are effective against damping-off, it does not address the

physiological problem of the hard seed coat. Repeated use of metalaxyl could also result in the development of resistant strains of the pathogen. The problems outlined above warrant the development of alternative control measures such as physiological disease control, cultural control, and integrated disease control which will be in agreement with sustainable and environment friendly agricultural practices.

In recent years, there has been growing interest in physiological disease control by application of seed priming techniques. Solid Matrix Priming (SMP) is one such seed priming technique developed by John Eastin (1990) to prime the seeds for greater seedling vigor and uniformity. SMP has been shown to alleviate the stand establishment problems caused by erratic germination, soil crusting, and damping-off diseases in several vegetable crops (Harman et al., 1988; Rush, 1991; Rush, 1992; Callan etal., 1990; and Kahn et al., 1992).

In Solid Matrix Priming, a solid carrier is used as an osmoticum to regulate the imbibition of water by the seeds. Solid matrix primed seeds are allowed to imbibe enough water so as to complete the pregermination process but not radical emergence. SMP differs from osmopriming in that a solid carrier is used as an osmoticum to regulate the imbibition of water by the seeds.

Several researchers have developed innovative control measures to tackle the problem of stand establishment and

damping-off losses in a number of vegetable crops. Strategies such as integration of SMP with inexpensive fungicides (Khan et al., 1992; Baird et al., 1994; and Parera et al., 1994), integration of SMP with biocontrol agents (Harman et al., 1988, 1989 and Callan et al., 1990), integration of osmopriming with fungicides (Osburn et al., 1989) and integration of SMP with irrigation practices (Rush, 1993) etc have given good results.

Even though erratic germination of okra has been a problem from many years (Anderson, 1960) there is not much information on the application of seed treatments such as SMP, chemopriming (integration of SMP with a fungicide/ fungicides) and biopriming (integration of SMP with biocontrol agent/agents) for improving seed germination and uniformity of okra seeds. Research on application of various seed priming techniques should help us to improve the germination rate and uniformity of okra seeds and reduce the damping-off disease losses. The main objectives of this study were

1. To evaluate ten different solid carriers for their efficacy as SMP agents to improve the rate of okra seed germination speed and vigor.
2. To develop SMP, chemopriming and biopriming protocols for okra seeds.
3. To identify the most common damping-off pathogens of okra from field soil and to assess their pathogenicity.

LITERATURE CITED

Abdelfattah, M. A., I. M. Abdalla, and A. A. Abd El-Hafez. 1972. Germination of okra seeds as affected by different treatments. Alexandria Journal of Agricultural Research 20:289-298.

Anderson, W. H., R. I. Carolus, and D. P. Watson. 1960. The germination of okra seed as influenced by treatment with acetone and alcohol. Proceedings of the American Society of Horticultural Science 62:427-432.

Baird, R. E., C. Nankam, P. F. Moghaddam and J. K. Pataky. 1994. Evaluation of seed treatments on shrunken-2 sweet corn. Plant Disease 78:317-821

Callan, N. W., D. E. Mathre, and J. B. Miller. 1990. Bio priming seed treatment for the biological control of *Pythium ultimum* reemergence damping-off in sh2 sweet corn. Plant Disease 74:368-372.

Edmond, J. B., and W. J. Drapala. 1960. Studies of the germination of okra seed. Mississippi Agricultural Experiment Station Technical Bulletin 47.

Eastin, J. A. 1990. Solid matrix priming of seeds. U.S. patent 4,912,874.

Grand, L. F. 1985. North Carolina plant disease index. North Carolina Agric. Res. Serv. Tech. Bull. 240:1-157.

Harman, G. E., and A. G. Taylor. 1988. Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78:520-525.

Harman, G. E., A. G. Taylor and T. E. Stasz. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Disease 73:631-637.

Khan, A. A., G. S. Abawi and J. D. Maguire. 1992. Integrating matriconditioning and fungicidal treatment of table beet seed to improve stand establishment and yield. Crop Sci. 32:231-237.

Marsh, L. 1992. Emergence and seedling growth of okra genotypes at low temperatures. HortScience 27:13101312.

Marsh, L. 1993. Moisture affects cowpea and okra seed emergence and growth at low temperatures. HortScience 28: 774-777.

Osburn, R. M., and M. N. Schroth. 1989. Effect of osmopriming sugarbeet seed on germination rate and incidence of *Pythium ultimum* damping-off. Plant Disease 73:21-24.

Parera, C. A., and D. J. Cantliffe. 1994. Presowing seed treatments to enhance supersweet sweet corn seed and seedling quality. HortScience 29:277-278.

Rush, C. M. 1991. Comparison of seed priming techniques with regard to seedling emergence and pythium damping-off in sugar beet. Phytopathology 81:878-882.

Rush, C. M. 1992. Stand establishment of sugar beet seedlings in pathogen-infested soils as influenced by cultivar and seed-priming technique. Plant Disease 76: 800-805.

Rush, C. M., and K. M. Vaughn. 1993. Effect of irrigation,soil matric potential,and seed priming on sugarbeet seed germination and damping-off caused by *Aphanomyces cochlioides.* Phytopathology 83:202-206.

Standifer, L. C., P. W. Wilson and A. Drummond. 1989. The effects of seed moisture content on hardseededness and germination in four cultivars of okra *(Abelmoschus esculentus(L)* Moench). Plant Varieties and Seeds 7:149-154

CHAPTER 2

SOLID MATRIX PRIMING OF OKRA SEEDS AS INFLUENCED BY MATRIX, WATER CONTENT AND NUMBER OF PRIMING DAYS

ABSTRACT

Okra seeds of the cultivar Clemson Spineless were solid matrix primed to improve germination and reduce damping-off problems. Ten solid carriers were evaluated for their ability to improve seed vigor of okra seeds at 25 C in laboratory studies. Solid carriers SA 500 (Super Absorbent) and CCA (Clay Carry-all) consistently increased ($P<0.05$) seed vigor in comparison to unprimed seeds. There were several significant ($P< 0.05$) interactions between priming agent, water content of the priming agent and priming time. In general,SA 500 and CCA performed better at water contents of 40% and 50% than the others. Water contents of 50% required fewer priming days, whereas water contents of 30% and 40% required more priming time to achieve the best priming. Therefore, SA 500 and CCA are recommended as potential priming agents for improving the seed vigor of okra seeds.

The cultivated land area planted to okra in Oklahoma is about 650 acres with a value of $ 2 million annually (Dr. J. Motes, *personal communication).* Delayed and erratic emergence is a serious problem in okra (Anderson et al; Edmond and Drapala, 1960; Abdelfattah et al., 1972; Standifer et al., 1989; and Marsh, 1992,1993) with regard to fertilizer utilization, post-emergence weed control and uniform harvesting. The hard seed coat of okra is a major physiological constraint to uniform stand establishment and performance (Standifer et al., 1989 and Marsh, 1992,1993). Okra seeds also have thick seed coats and contain more lipids than other vegetable seeds (Marsh, 1993).

Several researchers have attempted to overcome this problem by using techniques such as scarification with sulfuric acid for two hours (Standifer et al., 1989) and increasing the moisture content of seeds by placing them in moistened vermiculite (Marsh,1993) with limited success. Recent work (Marsh, 1992) has shown that field evaluations of 39 okra genotypes over three years revealed that all genotypes suffered from inconsistent seedling emergence in cold soils. It was suggested that if okra seeds were hydrated to an increased moisture content of about 50% then seed emergence in the field would be improved. The work of Standifer (1989) has suggested that hardseededness in okra is reversible and that seed moisture is an important factor in reducing seed coat hardness.

Recently, there is considerable interest in presowing seed treatment techniques such as Solid Matrix Priming (SMP) to improve the germination of the vegetable seeds. SMP has been highly effective in improving emergence and stand establishment of many crops (Khan et al, 1990; Rush, 1991,1992,1993;and Parera et al., 1993). In SMP,a solid carrier is used as an osmoticum to regulate the imbibition of water by the seeds.

The work of Harman and Taylor (1988) has suggested that the choice of the carrier used for SMP has a significant effect on the performance of seeds with regard to integration of biocontrol organisms in the priming agent. They also suggested that the priming agents differ in their efficacy because of their difference in pH and chemical composition. The objective of this study was to evaluate several priming agents at different water contents and priming times to improve the germination and vigor of the okra seeds. To our knowledge, this is the first report of the evaluation of SMP of okra seeds.

MATERIALS AND METHODS

Ten different SMP agents (Table 1) were screened for their ability to improve the germination and vigor of okra (CV. Clemson Spineless) seeds under lab conditions at 25°C.

The following protocol was used to screen all the ten

priming agents. The ratio of priming agent to seed (w/w) was 3:1. Weighed amounts of priming agents were placed in a polyethylene bags (4 inches X 2 inches) and sterile water was added to achieve 30%, 40% and 50% moisture content of the priming agent. The agent and the water were mixed thoroughly and ten okra seeds were added,the bags were sealed and incubated for 3,4,5, and 6 days at 25 C. Ten okra seeds (.6gms) were added to 1.8 gms of priming agent to achieve 30%,40% and 50% water contents respectively. For each priming agent and water content, a total of three replications of ten seeds each were done. At the end of the respective priming periods the seeds were separated from the priming agent by sieving. Ten seeds from each bag were placed in a petri dish (100mm X 15mm) lined with two layers of filter paper. Seeds were allowed to dry overnight on a lab bench at room temperature and the following day 5 ml of sterile water was added to all petri dishes. Germination counts were taken daily for 7 days. A seed was considered germinated when the radical tip protruded clearly from the seed. Untreated seeds served as the control. The experiment was repeated once. An index of seed vigor (Malvasi et al., 1985), expressed as Vigor index, was then calculated:

$$V\ I = \frac{G1 + G2 \ldots\ldots + GL}{D1 + D2 \ldots\ldots + DL}$$

V I = Vigor Index

G1 = Number of germinants (First count)

G2 = Number of germinants (Second count)

GL = Number of germinants (Last count)

D1 = Number of days to first count

D2 = Number of days to second count

D3 = Number of days to last count

Higher values of Vigor Index indicate better seed vigor. All data from the experiments were converted to get the vigor index value. Higher values of vigor index indicate better seed vigor. Analysis of variance tests were run and t-tests were used to compare all possible interactions between the main effects of matrix, water content, and priming time.

Results and Discussion

Of the ten priming agents tested (Table 1), Super Absorbent (SA 500) and Clay Carry-all (CCA) significantly improved ($P<0.05$) seed vigor when compared with the unprimed seeds in both experiments (Table 2). There were significant interactions ($P<0.05$) between matrix, water content and priming time (Table 2). SA 500 at 40 % water content for 4 and 6 days of priming time and at 50 % water content for 3 and 4 days priming time, gave significant increases in seed vigor in both the experiments. Priming in CCA at 40 % water

content for 6 days or 50 % for 5 days gave significant increases in seed vigor in both the experiments. Both SA and CCA performed well at water contents of 40% and 50%. Criteria such as capability to improve vigor of seeds, nontoxicity of priming agent to okra seeds, water holding capacity, friable nature of priming agent at various moisture levels, and ease of separation of seeds following priming were used to evaluate the relative efficacy of the priming agent. Pyrax and Agar Dust (Table 2) were showing some promise at certain levels but were eliminated as potential priming agents because they were encouraging growth of bacteria that was resulting in the seed decay and death. The remaining six priming agents (Table 2) did not result in consistent increases in seed and were also not recommended for future use.

Matthews and Powell (1986) suggested that seed vigor of the sown seeds is the major physiological constraint on the field performance of vegetable seeds. Seed vigor is defined as "that condition of active good health and natural robustness in seeds which, upon planting, permits germination to proceed rapidly under a wide range of experimental conditions" or "the potential for rapid germination and fast seedling growth under general field conditions". The results of our experiments have clearly demonstrated that solid matrix priming has the potential to improve the vigor of okra seeds. The "invigorating effect"

achieved by SMP on the okra seeds should have a positive effect on germination rate, particularly where the crop is direct seeded. We found that when okra seeds were primed at water contents of 40% and 50%, germination rate was improved significantly. These result are in agreement with the work of Marsh (1993) which suggested that a higher water content would help in improving the okra seed germination. We also found that the success of SMP was dependent on the priming agent used and the priming time. This shows the critical nature of the SMP technique and, therefore, we agree with the suggestion of Rush (1991) that a preliminary testing is required to determine the best combination of variables (i.e priming days, priming agent and water content).

The differences in the performance of the priming agents tested in this study could be attributed to their differences in chemical composition and pH values. It was interesting to note that the pH values of the two successful priming agents of nearly 7.0 (SA pH of 7.0 and CCA pH of 7.4) (Table 3,4) corresponds to the optimum soil pH for okra crop production of 7.0. The pH of other priming agents was not 7.0. For example, the pH of Agrolig, which did not perform well in this study, was 4.1 which is acidic and apparently okra is not tolerant to the acidic pH of this priming agent. This information is useful for future screening studies of priming agents for improving the seed vigor of a particular crop. It may be possible to eliminate

priming agents whose pH values are very different from those required for the optimum growth of the crop.

The potential applications of solid matrix priming of okra seeds are diverse. Apart from increasing the vigor of seeds, solid matrix priming may provide a delivery system for selective fungicides and biocontrol organisms to control various soilborne pathogens. Okra is very sensitive to cool temperatures and solid matrix priming could be used as a presowing seed treatment to improve the okra seed emergence and vigor in cold soils.

The successful priming agents in our study, namely SA 500 and CCA, are both noninjurious to okra seeds, friable, and can easily be removed from seeds. In conclusion we recommend SA 500 (Table 3) and CCA (Table 4) as promising priming agents for improving the vigor of okra seeds which otherwise gives erratic seed germination.

ACKNOWLEDGMENTS We would like to thank Dr. Kahn for providing the okra seeds. We also wish to thank Dr. Claypool and Luguang Wu for statistical advice and help. This research is funded by Oklahoma Center for the Advancement of Science and Technology (OCAST) grant.Project number AR2-055.

Table 1. Solid matrix priming agents evaluated for improving vigor of okra seed.

#	Priming agent	Chemical name	Characteristics	Source
1.	Carry-all 24/28C	Fullers earth	• Granular • Friable • Superdried	American Colloid Company
2.	Biodac	Nonclay agricultural carrier	• Bead like particles	Granulation tech. Inc.
3.	Super Absorbent	Fullers earth	• Granular ground clay	Balcones mineral Inc.
4.	Clay 16/30	Southern clay	• Granular	Edward Lowe indus--tries Inc.
5.	Pyrax ABB	Pulverized Pyrophyllite	• Powder	Industrial Minerals and Chemicals
6.	Agrolig (ESC)	-	• Fine powder	American colloid Company
7.	Agrolig (G)	-	• Granular	American Colloid Company
8.	Cat Litter 500m	-	• Fine	Walmart
9.	Cat Litter 1.0m	-	• Very Fine	Walmart
10.	Agar Dust	-	-	-

Table 2. Vigor index of priming agents at 25°C

PRIMING AGENT	WATER CONTENT	PRIMING TIME (DAYS)	VIGOR INDEX	
			EXPT 1	EXPT 2
UNPRIMED (CONTROL)			5.7	7.7
SA 500	30	3	8.5	17.7
		4	9.8	7.5
		5	13.4	11.0
		6	16.9	13.9
	40	3	9.0	17.3
		4	11.1	15.7
		5	14.0	11.3
		6	14.0	15.0
	50	3	10.4	14.9
		4	13.7	19.1
		5	11.3	8.6
		6	13.9	7.8
CCA	30	3	5.1	12.8
		4	5.5	8.6
		5	6.5	6.8
		6	8.1	5.6
	40	3	5.8	13.6
		4	8.4	8.0
		5	7.1	7.4
		6	9.2	15.7
	50	3	7.1	16.1
		4	6.2	15.1
		5	14.3	17.2
		6	15.4	9.8
Agardust	30	3	15.2	15.3
		4	7.9	7.9
		5	7.2	7.2
		6	5.4	5.5
	40	3	13.4	13.4
		4	8.4	8.4
		5	9.6	9.6
		6	9.9	9.9

PRIMING AGENT	WATER CONTENT	PRIMING TIME (DAYS)	VIGOR INDEX	
			EXPT 1	EXPT 2
	50	3	18.5	14.5
		4	8.6	8.6
		5	6.2	6.2
		6	12.9	12.9
Agrolig	30	3	9.8	9.8
		4	7.3	7.4
		5	7.2	7.2
		6	10.5	10.5
	40	3	13.2	13.2
		4	8.4	8.4
		5	6.5	6.5
		6	8.5	8.5
	50	3	5.0	12.7
		4	7.1	7.0
		5	7.0	5.3
		6	6.6	9.0
Agr_Gra	30	3	6.1	13.6
		4	6.9	7.6
		5	5.7	7.4
		6	6.1	7.2
	40	3	9.0	14.4
		4	4.5	11.2
		5	7.2	6.9
		6	9.0	12.5
	50	3	6.3	15.8
		4	7.1	13.7
		5	9.0	11.3
		6	10.9	12.0
Biodac	30	3	8.3	13.0
		4	7.5	9.1
		5	7.2	5.7
		6	8.3	10.1
	40	3	7.1	12.6
		4	9.7	8.4
		5	7.6	7.1
		6	7.5	12.3

PRIMING AGENT	WATER CONTENT	PRIMING TIME (DAYS)	VIGOR INDEX	
			EXPT 1	EXPT 2
	50	3	6.2	16.5
		4	8.0	8.4
		5	8.7	10.9
		6	14.1	12.8
CL10	30	3	4.7	12.7
		4	5.0	6.2
		5	5.6	7.8
		6	7.9	8.7
	40	3	4.6	9.8
		4	5.8	9.0
		5	7.7	8.2
		6	7.9	9.5
	50	3	5.7	13.2
		4	8.6	7.5
		5	9.2	9.3
		6	5.4	12.9
CL500	30	3	5.7	15.0
		4	6.6	6.2
		5	6.0	7.1
		6	5.9	9.0
	40	3	6.3	15.8
		4	8.5	9.8
		5	4.3	9.2
		6	9.0	9.3
	50	3	5.7	15.4
		4	10.1	10.3
		5	8.0	7.4
		6	10.1	5.8
Clay1630	30	3	7.4	12.5
		4	6.0	9.0
		5	9.1	6.0
		6	7.2	8.1
	40	3	8.6	11.1
		4	7.3	9.0
		5	5.9	6.9

PRIMING AGENT	WATER CONTENT	PRIMING TIME (DAYS)	VIGOR INDEX	
			EXPT 1	EXPT 2
		6	7.5	15.6
	50	3	8.3	14.8
		4	8.2	10.7
		5	8.8	11.2
		6	12.8	12.9
Pyrax	30	3	7.3	11.3
		4	10.0	12.2
		5	6.0	7.8
		6	22.2	0.0
	40	3	11.5	19.3
		4	15.7	12.6
		5	0.0	6.6
		6	0.0	0.0
	50	3	9.8	18.4
		4	4.8	0.0
		5	0.0	0.0
		6	0.0	0.0
LSD			3.5	6.4
Significance				
Matrix (M)			***	***
Water Content (WC)			NS	**
Priming Days (PD)			**	***
M X WC			***	**
M X DP			***	***
WC X DP			NS	NS
M X WC X DP			***	*

NS, ***, **, * Notsignificant or Significant at P<0.0001, 0.01, or 0.05, respectively
SA500 = Super Absorbent;CCA = Carry-all; Agrolic = Agrolig (ESC); Agr_Gra=Agrolig; CL10 = Cat Litter 1.0m; CL500 = Cat Litter 500m; Clay1630 = Clay 16/30; Pyrax = Pyrax ABB

Table 3. Characteristics of Super Absorbent solid matrix medium.

1. Trade name	Absorb-n-dry
2. Family	Clay (Montmorillonite type)
3. Character	Calcined ground clay
4. pH	7
5. Solubility	None
6. Stability	Very stable
7. Absorbency	90 % by weight (water) 96 % by weight (oil)
8. Usage	Material should be kept dry until use so that the effectiveness is at its fullest. Moisture starts the product to react.

Table 4. Characteristics of Carri-all 24/48C solid matrix medium.

1.	Liquid holding capacity	29.5 %
2.	Water absorbtivity	112 %
3.	Attrition resistance	87 %
4.	Free moisture	1 %
5.	pH	7.4
6.	Usage	For use in carrying a variety of active materials such as pesticides and fertilizers. Liquid holding capacity is good with flexibility for custom tailoring this property per application requirement.

LITERATURE CITED

Abdelfattah, M. A., I. M. Abdalla, and A. A. Abd El-Hafez. 1972. Germination of okra seeds as affected by different treatments. Alexandria Journal of Agricultural Research 20:289-298.

Anderson, W. H., R. I. Carolus, and D. P. Watson. 1960. The germination of okra seed as influenced by treatment with acetone and alcohol. Proceedings of the American Society of Horticultural Science 62:427-432.

Edmond, J. B., and W. J. Drapala. 1960. Studies of the germination of okra seed. Mississippi Agricultural Experiment Station Technical Bulletin 47.

Harman, G. E., and A. G. Taylor. 1988. Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78:520-525.

Khan, A. A., G. S. Abawi and J. D. Maguire. 1992. Integrating matriconditioning and fungicidal treatment of table beet seed to improve stand establishment and yield Crop Sci. 32:231-237.

Malavasi, D. M., Stafford and D. P. Lavender. 1985. Stratifying, partially redrying and storing douglas-fir seeds: effects on growth and physiology during germination. Ann. Sci. For 42 :371-384.

Marsh, L. 1992. Emergence and seedling growth of okra genotypes at low temperatures HortScience.27:13101312.

Marsh, L. 1993. Moisture affects cowpea and okra seed emergence and growth at low temperatures. HortScience 28:774--777.

Matthews, S.., and A. A. Powell. 1986. Environmental and physiological constraints on field performance of seeds. HortScience 21:1125-1128.

Parera, C. A., and D. J. Cantliffe. 1994. Presowing seed treatments to enhance supersweet sweet corn seed and seedling quality. HortScience 29: 277-278.

Rush, C. M. 1991. Comparison of seed priming techniques with regard to seedling emergence and pythium damping-off in sugar beet. Phytopathology 81:878-882.

Rush, C. M. 1992. Stand establishment of sugar beet seedlings in pathogen-infested soils as influenced by cultivar and seed-priming technique. Plant Dis 76:800-805.

Rush, C. M., and K. M. Vaughn. 1993. Effect of irrigation,soil matric potential,and seed priming on sugar beet seed germination and damping-off caused by *Aphanomyces cochlioides.* Phytopathology 83:202-206.

Standifer, L. C., P. W. Wilson and A. Drummond. 1989. The effects of seed moisture content on hardseededness and germination in four cultivars of okra *(Abelmoschus esculentus(L)* Moench). Plant Varieties and Seeds 2:149-154.

CHAPTER 3

INTEGRATION OF SOLID MATRIX PRIMING AND FUNGICIDAL TREATMENT OF OKRA SEED TO IMPROVE STAND ESTABLISHMENT AND REDUCE PREEMERGENCE DAMPING-OFF

ABSTRACT

Two field trials were conducted to evaluate the efficacy of solid matrix priming technique alone, or in combination with fungicides (Thiram + Carboxin), biocontrol agent (*Trichoderma harzianum*) and a polymer seed coating (carboxymethylcellulose) to improve seed emergence and reduce damping-off of okra (clemson spineless) in field soil. Chemoprimed seeds gave more uniform and faster emergence compared to the untreated control at both the field trials. Within three days, 92% of the chemoprimed seeds at Stillwater and 72% of the chemoprimed seeds at Bixby had already emerged. The mean rate of emergence of chemoprimed seeds was significantly lower ($P<0.05$), signifying faster emergence at both locations compared to untreated control. Chemoprimed seeds significantly improved (P,0.05)the seed vigor at both locations compared to fungicide treatment or primed seed alone at both locations. There were no significant differences ($P<0.05$) between the yield of the six treatments at both locations. Pathogenicity tests have revealed that *Pythium ultimum* was

the most pathogenic damping-off causing agent in the field soil. *Rhizoctonia spp.* and *Fusarium spp.* were also pathogenic. A combination seed treatment of solid matrix priming and fungicides (Thiram + Carboxin) is recommended to improve okra seed vigor and reduce soilborne disease problems under field conditions.

The cultivated land area of okra in Oklahoma is about 650 acres and at a value of $ 2 million annually (Dr. J. Motes, *personal communication).* Erratic germination in okra results in delayed seedling emergence and presents serious problems with fertilizer utilization, post-emergence weed control and uniform harvesting (19). Factors such as hard seed coat and pre-emergence damping-off (particularly by *Pythium spp)* contribute to the erratic germination in okra. Environmental constraints such as inadequate or excessive moisture, low temperatures, soil crusting of silt soils, poorly prepared seed beds also contribute to this problem. Growers cannot control the environmental conditions however, it is possible to control the vigor of the seed in order to improve the probability of successful stand establishment. Vigor of the sown seeds is the major physiological constraint to the field performance of vegetable seeds such as okra (14). Any seed treatment that increases vigor should lead to more consistent stand establishment. Seed priming is one such potential physiological seed treatment

technique which could be used to increase the field performance of vegetable seeds such as okra. At the same time priming may reduce damping-off losses because of the rapid growth and emergence of the primed seeds(3,18).

Seed hydration techniques have been tried as early as 1919 (10) to improve the rate and uniformity of the seeds. Osmotic priming is one kind of seed priming where a osmotic solution such as potassium nitrate or polyethylene glycol is used to regulate the imbibition of water. The water potential of the osmoticum is adjusted in such a manner so that enough water is imbibed by the seed to complete its pre-germination metabolic activities, but prevents the emergence of the radical. Osmotic priming has been shown to improve the rate and uniformity of the seed germination and reduce the damping-off losses (3,15). However, its utility is limited by such factors such as lack of aeration to seeds due to the viscous nature of polyethylene glycol, requirement of large volumes of solutions, and problems in temperature control of the osmoticum. To overcome this problem, a new method of priming termed Solid Matrix Priming (SMP) was developed by John Eastin (6) (US patent 4,912,874). In SMP, a solid medium is used to regulate the imbibition by the seeds. Solid matrix priming alone (18) or in combination with fungicides (2,9) or biological control agents (4,7,8) has been successfully shown to improve the rate and uniformity of vegetable seeds and reduce

damping-off losses.

Even though erratic germination has been reported as a problem in okra since 1960 (1), little research has been conducted on evaluation of various preconditioning treatments such as SMP, SMP plus fungicides (Chemopriming) and SMP plus biocontrol agents (Biopriming). The work of Standifer et al (1989) has suggested that the problem of a hard seed coat of okra is reversible and an increase in moisture content of the okra seed has beneficial effects in emergence and uniformity under field conditions. Recently, Marsh (1992,1993) evaluated 39 okra genotypes under cold soil conditions in the field over a three year period and showed inconsistent emergence and stand uniformity. He suggested that increasing the moisture content of seeds to 52 % might improve seed germination in the field.

The objectives of this study were 1) To integrate solid matrix priming with the recommended fungicidal seed treatments for okra seeds to improve the seed vigor and reduce pre-emergence damping-off. 2) To compare the relative efficacies of various seed treatment combinations such as chemopriming (SMP + fungicides), biopriming (SMP + biocontrol agent) and SMP + polymer seed coating(Carboxy Methyl Cellulose) (CMC). 3) To identify the most common fungi associated with okra seedlings and to assess their pathogenicity in the field soil .

Chemopriming. The following protocol was used for achieving chemoprimed seeds. Fifty okra seeds of the cultivar Clemson Spineless treated with the fungicide seed treatment of thiram plus carboxin were placed in a self-sealing polyethylene bag containing the CCA priming agent at 50% water content (- 0.7 Mpa water potential). The ratio of priming agent to seed (wt/wt) was 3:1. The seeds (Fifty) were mixed with the priming agent (8.7gms) to which 4.35ml of water was added and the bag was incubated at room temperature. Four replications of 50 seeds each were prepared in the above procedure. After incubation for 3 days, the chemoprimed seeds were separated from the matrix by sieving. The seeds were allowed to dry overnight and were then transferred to small brown paper bags for transport to the field.

Bioprimed and polymer coated seeds. Fifty untreated okra seeds cv. Clemson Spineless were primed as described above in the solid matrix priming protocol. After priming, the seeds were dried overnight and then coated with a biocontrol agent (*Trichoderma harzianum* OK 110, 0.1 gm added w/v with 1 % carboxymethyl cellulose) suspended in 1 % CarboxyMethyl Cellulose (CMC). The resultant mixture of biocontrol agent and CMC had approximately 10^6 CFU/ml of *Trichoderma* . Four replications of 50 seeds each were prepared as described

above. The seeds were dried under a laminar flow hood overnight (12 hours) and were transferred to small paper bags for transport to field. For preparation of polymer coated seeds, primed seeds were dried overnight and then suspended in 1 % Carboxymethyl cellulose and again dried overnight and bagged for transport to field.

Seed treatments. Six different seed treatments with four replications of 50 seeds each were used for the field experiments. The six seed treatments were: 1) Untreated seed 2) Untreated plus fungicide (thiram + carboxin) 3) Primed seed, no fungicide 4) Chemoprimed seed (primed + fungicide mix of thiram and carboxin) 5) Bioprimed seed (primed seed + *Trichoderma harzianum* OK 110 + 1% CMC) 6) Primed seed plus 1% CMC.

Field trials. Field trials were conducted at two locations to evaluate these six seed treatments. One trial was conducted at Oklahoma at Bixby, OK. The second field trial was located at the Plant Pathology Research Farm at Stillwater, Oklahoma. A completely randomized design was used at both locations. The plots were disked and fertilizer was applied according to standard agricultural practices for okra crop as recommended by Oklahoma State University soil testing laboratory. Each plot was 26 m deep and 20 m wide. There were 24 rows and each row was 5 m long and 1 m wide. The rows were separated by a spacing of 2 m. At both locations okra seeds were planted with a belt

seeder (operated manually) calibrated to seed at a depth of 4cm. Seeds were planted on May 17 at Stillwater and on May 19 at Bixby. Emergence was recorded daily at both the locations for eight days. Newly emerged okra seedlings were marked with plastic coffee stirrers when they started emerging. A seed was considered emerged when the hypocotyls were above the soil surface. 3-4 weeks after emergence a 3m long sub-data plot containing exactly 15 okra plants within each row in the plot was created by thinning seedlings. Sub-plots were harvested repeatedly by hand for yield data. Thus, any differences in yield after thinning could not be attributed to stand variation. About 14-15 picks were harvested by hand at both locations. The picks were done at a intervals of 2-3 days at both sites.

Isolation of fungi from seedling and soil samples. Diseased okra seedlings were collected randomly from the experimental plots along with surrounding soil. The seedlings were washed under running water for 5 min to remove all soil debris, rinsed with sterile water, blotted dry with sterile paper towels, and plated on Potato Dextrose Agar. Representative fungal colonies were hyphal tipped to selective medium for identification.

Isolation of Pythium spp. from soil. Soil samples were thoroughly mixed and air-dried before use. Samples from each seedling were processed separately. Two grams of soil from each soil sample were added to 38 ml of a dilution

suspension containing water agar (0.3%) and calcium chloride (0.368/100ml) adjusted to pH 5.5 with 1% phosphoric acid. This suspension was agitated and 1 ml was spread on the pythium selective medium (5). After 24 hr of incubation at 25 C in the dark, the surface of the agar in each petri dish was gently washed in a stream of water to remove soil and bacterial growth. Representative colonies were transferred to Potato Dextrose Agar for verification and speciation of *Pythium*. For the identification of *Pythium* spp., a grass blade water solution was prepared (20). Species were identified by using the key of Van der Plaats-Niterink (20).

Isolation of Rhizoctonia spp. from soil. Collected soil samples were thoroughly mixed and air-dried before use. Concentrated dilutions of five grams of soil in 0.25 % water agar was placed in wells in dishes of selective medium with wide mouthed pipetter (17).

Data analysis. The seedling emergence rate was expressed as the Mean Rate of Emergence (MRE)[MRE = $(N)+T_2N_2 + \ldots + T_nN_n$ / Total number of seedlings emerged, where N= total number of seedlings emerged and T= day number](15). An index of seed vigor (11), expressed as germination vigor index was also calculated:

$$V\ I = \frac{G1 + G2 \ldots\ldots + GL}{DI + D2 \ldots\ldots + DL}$$

VI = Vigor Index

G1 = Number of germinants (First count)

D1 = Number of days to First count

G2 = Number of germinants (Second count)

D2 = Number of days to Second count

GL = Number of germinants (Last count)

DL = Number of days to last count

All data from the experiments was converted to Vigor Index and MRE values. Then the data was subjected to analysis of variance and t-tests($P < 0.05$) were used to compare the six seed treatment means. Higher values of vigor index indicates better vigor and lower values of MRE indicates faster emergence. Stand counts were expressed as percent of the total number of planted seeds.

RESULTS

Field Experiments. In general, the stand at both Stillwater and Bixby was excellent because of the favorable growing conditions and little disease. At both locations, chemopriming was the best treatment combination (Table 5,6) throughout the emergence period (8 days). After the first eight days the stand had stabilized. Within two days at Bixby twice the number of chemoprimed seeds had emerged compared to untreated seeds. In general, similar trends were observed at both the locations. Seedlings of chemoprimed

seeds emerged more uniformly at both locations. Within three days chemoprimed seeds achieved 92% of total possible stand at Stillwater and 77% of total possible stand at Bixby. However, there were fewer differences between the treatments by the end of the eight days after emergence. Chemoprimed seeds had a significantly better three day stand count ($P<0.05$) than the untreated seed at Stillwater (Table 5). Emergence of seeds treated by priming alone was not greater when compared to unprimed, untreated control. Stand of bioprimed and polymer (CMC) coated seeds was not greater than any of the treatments at either locations. The mean rate of emergence of chemoprimed seeds was significantly lower ($P< 0.05$), signifying faster emergence than the untreated seeds at both locations (Table 5,6). Of the six treatments evaluated, chemopriming resulted in significantly greater ($P< 0.05$) seed vigor at both locations. However, the seed vigor of the primed seed alone was not significantly greater than fungicidal treated seeds. Yields were not significantly different at both trials (Table 7). Fungi recovered from seed and soil samples. A wide variety of fungi were isolated from seedling and soil samples (Table 8). *Pythium* spp. was the most predominant fungus isolated. Other damping-off pathogens such as *Rhizoctonia* spp. and *Fusarium* spp. were also isolated. Saprophytes such as *Rhizopus, Mucor spp. and Chaetomium* spp were isolated from seedling samples only. A *Trichoderma* isolate was also

recovered. Pathogenicity testing showed that *Rhizoctonia* spp. were weakly pathogenic and one isolate of *Pythium* spp. was found to be an very aggressive pathogen of seedlings (particularly at low temperatures). This isolate was identified as *Pythium ultimum*. Pathogenicity testing of *Rhizoctonia* spp. and *Fusarium* spp. has shown that these two isolates were weakly pathogenic and therefore were considered to be minor root pathogens for okra seedlings.

DISCUSSION

Recently several researchers have successfully integrated the benefits of solid matrix priming with fungicides in different crops (2,9,15,16). Our results have also demonstrated that it is possible to integrate the advantages of physiological seed treatments such as solid matrix priming with commercially available fungicide seed treatments for improving the seed vigor of okra seeds. We have also shown that inexpensive fungicides such as thiram and carboxin having activity against *Pythium* spp. and *Rhizoctonia* spp., could be combined with solid matrix priming resulting in an synergistic effect. We have termed the integration of solid matrix priming with fungicidal seed treatments as "chemopriming". Our results have shown that the seed vigor of chemoprimed seeds is even greater than the fungicide treated seed at two different locations varying in

soil types.

In our study, biopriming gave unsatisfactory results. Previous work by Callan et al (1990) and Harman et al (1988,1989) showed that solid matrix priming could be integrated with biocontrol agents to achieve improved emergence. Possible reason for failure of biopriming seed treatments in our study could be the differences in the biopriming protocols. In our study we have first primed the seed (with the intention of softening the seed coat first) and then biocontrol agent was added to the seed with the help of the carboxy methyl cellulose sticker. Perhaps addition of biocontrol agent before priming or during priming as done in Harman's (1988,1989) and Callan's (1990) study could be beneficial. Current efforts in this laboratory are directed to achieve successful biopriming by changing the protocol. The study of Baird's group has also shown that solid matrix priming could be integrated with a polymer seed coating such as Ongard^{R}. In our study, a polymer seed coating was not of any benefit to improve the seed vigor of okra. This could be possibly explained because of the differences in the seed microstructure of okra to sweet corn and also to the differences in the polymer seed coating used in our study.

This study has shown that *Pythium ultimum* was one of the primary damping-off pathogen of okra while *Rhizoctonia* spp. and *Fusarium* spp. are also capable of causing

damping-off problems. Because of the small sample size collected from the field plots we were unable to perform any meaningful statistical analysis to compare the treatment effects with isolation frequency of these fungi. Damping-off is an endemic problem at Bixby as vegetables have been grown there since the early 1900's. Therefore, it was not unexpected to observe that the stands at Bixby were less when compared to stands at Stillwater which had less disease pressure.

A possible explanation for the superior performance of chemoprimed seeds over other seed treatments in this study may be because of the " synergistic effect " of the integration of physiological disease control with fungicidal seed treatment. Another explanation for the better performance of the chemoprimed seeds could be the higher rate of emergence of this treatment over other seeds resulting in an good example of apparent resistance by " disease escape "

Because of the fact that a diversity of soilborne pathogens (Table 8) could be responsible for causing seedling mortality in okra, a broad spectrum, multicomponent seed treatment including seed priming is suggested as a control strategy for good stand establishment of okra seeds in field soil. Since chemoprimed okra seeds successfully addresses both the physiological (softening seed coat) and pathological (reduction of soilborne diseases) seed problems

of okra, it is recommended as a viable control strategy for successful okra crop production.

Table 5. Effects of seed treatments on stands(%), Mean Emergence Rates and Vigor Index of okra planted at Stillwater.

Treatment[x]	3 day stand(%)	8 day stand(%)	Vigor Index	MER (days)
Chemoprimed	91.5 a[y]	88.5a[z]	54.3a[m]	1.9[n]
Primed	84.5 b	80.5b	45.8bc	2.1
Treated	91.5 a	87.5a	48.6b	2.0
Untreated	84.1 b	87.5a	44.8bc	2.2
CMC	81.5 b	86.0a	43.4bc	2.2
Bioprimed	80.0 b	77.0b	42.5c	2.2

[x]Except for untreated control all the other were subjected to solid matrix priming with clay carri-all at 25 C.
CMC = Carboxy Methyl Cellulose.
[y],[z] Means followed by the same letter are not significantly different (PS 0.05) according to Duncan's multiple range test.
[m]Vigor Index.LSD(P<O.O5)=5.3
[n]MER = Mean Emergence Rate (Days). LSD (PS 0.05) = 0.2

Table 6. Effects of seed treatments on stands (%) and Mean Emergence Rates of okra at Bixby

Treatment[x]	3 day stand(%)	8 day stand(%)	Vigor Index	MER (days)
Chemoprimed	77.5 a[y]	85.0a[z]	48.4a[m]	2.4[n]
Primed	72.0 ab	81.0ab	38.5b	2.6
Treated	67.0 abc	83.5a	33.0bc	3.2
Untreated	71.0 abc	77.0ab	33.1bc	3.1
CMC	60.0 bc	69.5b	37.0bc	2.7
Bioprimed	59.0 c	70.0b	30.4c	3.2

[x]Except for untreated control all the other were subjected to solid matrix priming with clay carri-all at 25 C.
CMC = Carboxy Methyl Cellulose.
[y,z] Means followed by the same letter are not significantly different (PS 0.05) according to Duncan's multiple range test.
[m]Vigor Index. LSD ($P<0.05$)=6.9
[n]MER = Mean Emergence Rate (Days). LSD (PS 0.05) = 0.6

Table 7. Effects of seed treatments on marketable fruit number and weight of Okra at Stillwater and Bixby.

Treatment[x]	Stillwater		Bixby	
	MFW (grams)	MFN	MFW (grams)	MFN
Chemoprimed	341.3	24.5a	416.6	32.0a
Primed	314.3	23.8a	430.1	32.8a
Treated	321.9	24.0a	394.4	30.3a
Untreated	323.2	23.4a	415.6	31.5a
CMC	322.7	24.8a	406.7	31.0a
Bioprimed	341.3	25.9a	404.3	31.2a
LSD(P<0.05)	34.1	2.94	33.4	2.64

[x]Except for untreated control all the other were subjected to solid matrix priming with clay carri-all at 25 C.
CMC = Carboxy Methyl Cellulose.
MFN = Marketable Fruit Number.
MFW = Marketable Fruit Weight.(Fresh weight in grams).
MFN and MFW values represent the mean of 60 observations at Stillwater and 52 observations at Bixby.

Table 8. Fungi commonly isolated from seed and soil samples.

Fungi	Source[x]	Pathogen[y]
Pythium ultimum	3	+++
Pythium spp.	1	NP
Rhizoctonia solani	1	+
Fusarium spp.	1	+
Rhizhopus spp.	2	NT
Mucor spp.	2	NT
Chaetomium spp.	2	NT
Trichoderma spp.	3	NT

[x]Soil isolation = 1 ; Seed isolation = 2 and 1 + 2 = 3.
[y]Aggresive pathogen = +++; Moderately aggressive = ++; Weakly aggressive = + ; NP= Non Pathogen and NT= Not Tested.

LITERATURE CITED

1. Anderson, W. H., R. I. Carolus, and D. P. Watson. 1960. The germination of okra seed as influenced by treatment with acetone and alcohol. Proceedings of the American Society of Horticultural Science 62:427-432.

2. Baird, R. E., C. Nankam, P. F. Moghaddam and J.K.Pataky.1994. Evaluation of seed treatments on shrunken2 sweet corn. Plant Disease 78:817-821.

3. Bradford, K. J. 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience 21:1105-1112.

4. Callan, N. W., D. E. Mathre, and J. B. Miller. 1990. Biopriming seed treatment for the biological control of *Pythium ultimum* preemergence damping-off in sh2 sweet corn. Plant Disease 74:368-372.

5. Conway, K. E. 1985. Selective medium for isolation of *Pythium* Spp. from soil Plant Disease 69:393-395.

6. Eastin, J. A. 1990. Solid matrix priming of seeds. U.S. patent 4,912,874.

7. Harman, G. E., and A. G. Taylor. 1988. Improved seedling performance by integration of biological control agents
at favorable pH levels with solid matrix priming. Phytopathology 78:520-525.

8. Harman, G. E., A. G. Taylor and T. E. Stasz. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Disease 73-631-637.

9. Khan, A. A., G. S. Abawi and J. D. Maguire. 1992. Integrating matriconditioning and fungicidal treatment of table beet seed to improve stand establishment and yield. Crop Sci. 32:231-237.

10. Kidd, F., and C. West. 1919. Physiological predetermination: the influence of the physiological condition of the seed upon the course of subsequent growth and yield. IV. Review of the literature. Chapter III. Annals of Applied Biology.

11. Malvasi, D. M., Stafford and D. P. Lavender. 1985. Stratifying,partially redrying and storage of douglas fir seeds: effects on growth and physiology during

germination. Ann. Sci. For.42(4):371-384.

12. Marsh, L. 1992. Emergence and seedling growth of okra genotypes at low temperatures. HortScience27:13101312.

13. Marsh, L. 1993. Moisture affects cowpea and okra seed emergence and growth at low temperatures HortScience 28:774-777.

14. Matthews, S. ., and A. A. Powell. 1986. Environmental and physiological constraints on the field performance of seeds. HortScience 21:1125-1128.

15. Osburn, R. M., and M. N. Schroth. 1989. Effect of osmopriming sugarbeet seed on germination rate and incidence of *Pythium ultimum* damping-off. Plant Disease 73:21-24.

16. Parera, C. A., and D. J. Cantliffe. 1994. Presowing seed treatments to enhance supersweet sweet corn seed and seedling quality. HortScience 29:277-278.

17. Ploetz, R. C., Mitchell, D. J., and Gallaher, R. N. 1985. Characterization and pathogenicity of *Rhizoctonia* species from a reduced-tillage experiment multicropped to rye and soybean in Florida. Phytopathology 75:833839

18. Rush, C. M. 1991. Comparison of seed priming techniques with regard to seedling emergence and pythium damping off in sugar beet. Phytopathology 81:878-882.

19. Standifer, L. C., P. W. Wilson and A. Drummond. 1989. The effects of seed moisture content on hardseededness and germination in four cultivars of okra *(Abelmoschus esculentus* (L) Moench). Plant Varieties and Seeds. 2:149-154.

20. Van der Plaats-Niterink. A. J. 1981. Monograph of the genus *Pythium* . Studies in Mycology No. 21. Central bureau voor Schimmelcultures, Baarn, Netherlands.

Vita

Mereddy Ranjit

Candidate for the Degree of

Master of Science

Thesis: INTEGRATION OF SOLID MATRIX PRIMING WITH CHEMICAL AND BIOLOGICAL SEED TREATMENTS TO IMPROVE OKRA SEED GERMINATION AND REDUCE PREEMERGENCE DAMPING-OFF BY PYTIHIUM ULTIMUM

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Hyderabad, India, December 28, 1968, the son of Dr. Satyanarayana Reddy M. and Neeraja Devi M.

Education: Graduated from Little Flower Junior College, Hyderabad, India, in March, 1986; received Bachelor of Science Degree in Agriculture from Andhra Pradesh Agricultural University at Hyderabad, India in August, 1991; Completed the requirements for the Master of Science degree with a major in Plant Pathology at Oklahoma State University in July 1995.

Professional Experience: Research Assistant and Teaching Assistant, Department of Plant Pathology, Oklahoma State University, August, 1992, to February,1995; Mycological Herbarium Curator, Department of Plant Pathology, Oklahoma State University, August, 1992, to February,1995; Diagnostic Clinic Training, Oklahoma State University, November,1994, to March,1995.

Awards: Finalist of the Phoenix Masters Student Award Sponsored by the Graduate Student Association,Spring,1995.

Professional Affiliations: American Phytopathological Society.