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I am submitting herewith a thesis written by Raymond E. McNew entitled "Effect of Three Foliar Fungicides on Germination, Emergence, and Internally-Borne Fungi in Seeds from Six Cultivars of Soybean." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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B

EFFECT OF THREE FOLIAR FUNGICIDES ON GERMINATION, EMERGENCE, AND INTERNALLY-BORNE FUNGI IN SEEDS FROM SIX CULTIVARS OF SOYBEAN

A Thesis

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ABSTRACT

The purpose of this study was to determine the efficacy of foliar applications of thiabendazole (Mertect 340 F), benomyl (Benlate 50 WP), and chlorothalonil (Bravo 6 F) in improving seed quality of six cultivars of soybean. Factors used to determine seed quality were germination, emergence, postemergence damping-off, and incidence of fungi associated with the seeds.

In the germination test, there were significant differences among cultivars, among treatments, and among replications. Germination of Forrest and Ring Around-501 (RA-501) was significantly higher than other cultivars. Thiabendazole gave a significantly higher mean germination than the other two fungicides, but was not significantly different from the control. Significant differences between replications were unexplained.

In the emergence test, Forrest and Essex had significantly higher means of emergence than other cultivars. Thiabendazole was significantly different from the control and chlorothalonil, but not from benomyl in increasing emergence. Differences between replications were less pronounced than in the germination test.

In the test for postemergence damping-off, Forrest had a significantly lower percentage of seedlings damped-off than the other cultivars. There were no significant differences between treatments or between replications.

There were no significant correlations between the incidence of fungi isolated and germination, emergence, postemergence damping-off, cultivar, treatment, and replication. *Diaporthe phaseolorum* var. *sojae* was found internally seedborne in 5.58% of seeds tested, and oospores of *Peronospora manshurica* occurred on 2.83% of the seeds. The mean percent of total fungi isolated was 19.58%.

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CHAPTER I

INTRODUCTION

The soybean, *Glycine max* (L.) Merrill, is one of the oldest crops in modern agriculture (37,63). The cultivated form of the soybean originated thousands of years ago in mainland China, and was disseminated through Korea into Japan (37). Today, soybeans are an important crop worldwide.

Soybean production and demand in the United States have risen dramatically in recent years. In 1979, soybeans were the number one cash crop, the number one cash export, and the number one crop in acres harvested for grain in the United States. Soybean acreage in the United States totaled 70.7 million acres in 1979, and production exceeded 53 million metric tons (3).

Soybean yields can be greatly reduced by losses due to diseases. In Tennessee alone, in 1979, the total disease loss was estimated to be 181.9 million bushels (2). At an average price of $6.25 per bushel, Tennessee farmers lost approximately $1,136,875,000 to disease organisms in one growing season. One method utilized to reduce yield losses due to diseases has been the use of high quality seed. Scott and Aldrich (61) listed six criteria for determining seed quality: (a) varietal purity; (b) germination and vigor; (c) mechanical purity and low percentage of inert matter; (d) low numbers of weed seeds; (e) few other crop seeds; and (f) uniformity of size.

This study dealt with the efficacy of three foliar fungicides for improving germination and vigor of seeds of six soybean cultivars by reducing the incidence of internally seedborne pathogens.

CHAPTER II

LITERATURE REVIEW

Fungicides

Application of foliar fungicides to prevent loss of viability or improve the quality of soybean seeds is a relatively recent development in plant disease control. Crittenden and Bloss (11) applied zineb, maneb, and tribasic copper sulfate to soybeans as foliar sprays. All three fungicides gave good control of *Cercospora kikuchii* (T. Matsu and Tomoyasu) Chupp (= *Cercosporina kikuchii* T. Matsu and Tomoyasu), the causal organism of purple seed stain of soybeans. The fungicides gave no control of *Diaporthe phaseolorum* (Cke. and Ell.) var. *sojae* Wehm. (= *D. sojae* Leh.); imperfect stage: *Phomopsis sojae* Leh., the causal organism of pod and stem blight of soybeans. Crittenden and Bloss later achieved control of both organisms with foliar sprays of zineb and tribasic copper sulfate applied when the soybeans were in the reproductive stage.

Prasartsee and Sinclair (57) sprayed soybean plants with mancozeb, thiophanate-methyl, chlorothalonil, thiabendazole, benomyl, and benomyl + mancozeb at 10 day intervals from 70 to 100 days after planting, at then-recommended rates. Seeds from the sprayed plants had a lower incidence of *D. phaseolorum* var. *sojae* than seeds from controls.

Bolkan and Cupertino (6) tested five fungicides applied as foliar sprays. Benomyl, captafol, thiabendazole, chlorothalonil, and thiophanate all reduced the occurrence of *P. sojae* internally seedborne, and increased in vitro germination of seeds from treated plants. Bolkan and Cupertino reported no phytotoxic effects from the fungicides.

Ilyas *et al.* (36) reported that foliar applications of benomyl resulted in seeds with higher germination rates and lower incidence of *D. phaseolorum* var. *sojae* than controls. Sprays of captafol, chlorothalonil, or mancozeb had no significant effect. Ilyas *et al.* observed that seeds from benomyl-sprayed plants had a higher occurrence of *Alternaria* spp. than seeds from other treated or untreated plants. Ellis (18,24) noted that *Alternaria* spp. were tolerant to methyl 2-benzimidazolecarbamate (MBC), the breakdown product of benomyl. Ross (60) found a generally inverse relationship between the incidence of *Alternaria* spp. and that of *P. sojae*, and postulated an antagonism between the two organisms in infected soybean seeds.

More research has been conducted on the effects of benomyl on internally seedborne fungi than on other fungicides. Benomyl was effective in increasing germination rates, decreasing the occurrence of *D. phaseolorum* var. *sojae* and other fungi, and improving seed quality when applied as a foliar spray (6,18,21,24,27,36,57,58,60, 62,70). Benomyl was especially useful in preventing the deterioration of seed quality related to delayed harvest (6,18,21,24,27,36, 57,58,60). The proportion of seedborne *D. phaseolorum* var. *sojae*

and other fungi increased and germination decreased with time after maturity of the plants and before harvest (4,12,18,24,41,43,60,75). Davis and Andrews (12) found that field conditions associated with harvest date appeared to have a greater effect on seed microflora than did fungicide treatments.

Ellis and Sinclair (23) reported that benomyl was hydrolyzed to methyl 2-benzimidazolecarbamate (MBC) in solution. MBC was absorbed through the stems of soybean plants and translocated in the apoplast. MBC also entered through pod walls, and was found in seed coats and cotyledons 24 hours after spraying. A bioassay for MBC was conducted on plates of potato-dextrose agar (PDA) seeded with spores of *Penicillium expansum* Link or *D. phaseolorum* var. *sojae*. A larger zone of inhibition developed around the seed coats than around the cotyledons, indicating a higher concentration of the fungicide in the seed coats. MBC became indetectable in the seeds in storage after harvest when the foliar spray was of low concentration. MBC activity decreased with time after the initial application, and appeared to have little residual effect.

Thiabendazole was effective in reducing the incidence of seedborne *D. phaseolorum* var. *sojae* (6,57,58,62), and in increasing in vitro germination of seeds from treated plants (6) when used as a foliar spray. The only conflicting report was made by Foor and Sinclair (27), who found that thiabendazole did not improve seed quality.

In 1971, Gray and Sinclair (30) used ^{14}C-labeled thiabendazole to observe systemic action of the fungicide in soybean seedlings. The labled thiabendazole was taken up and translocated in unaltered form to all above-ground plant parts. Gray and Sinclair found that the concentration of fungicide was highest in the cotyledons, epicotyl tissue, and in the roots.

Little research has been done on the effectiveness of chlorothalonil. Several researchers found that chlorothalonil was effective in reducing numbers of seedborne fungi, including *D. phaseolorum* var. *sojae* (6,57,58,62), and that the fungicide increased in vitro germination (6). Conflicting reports were made by Foor and Sinclair (27) and Ilyas *et al.* (36), who observed no improvement in seed quality when chlorothalonil was used as a foliar spray.

Direct seed treatment with fungicides has also been used to improve germination (10,20), emergence (20,71), and stand (56) of seeds infected by *D. phaseolorum* var. *sojae* and other internally seedborne fungi. Seed treatment has been used when seed lots were known to have a high percentage of infection or a low germination rate. Fungicides commonly applied as seed treatments have included captan (10,20,56), thiram (10,20), mancozeb, Uniroyal 1109 and 1110 (56), and benomyl (20). All treatments were effective, but Ellis *et al.* (20) found that benomyl penetrated the embryo of the seeds and was phytotoxic. Use of fungicidal seed treatments was only recommended for poor quality seed (10,56,72,73). In these lots, germination was increased by 2 to 27% over untreated samples (10),

and emergence was increased by at least 19% (71). Treatment of high quality seed was not significantly effective (9,56).

Seedborne Organisms

Sinclair (62) reported about 100 microorganisms associated with soybean seeds, including fungi, bacteria, and viruses. Not all of these are important sources of reduction in seed quality, but most have caused some quality loss under appropriate conditions. Only those organisms pertinent to this research have been included. Fungi which fall into this category are: *Alternaria* spp. (24,27,40,53,54, 62,63,67); *Aspergillus* spp. (13,19,33,40,62,63,67); *Chaetomium* spp. (53,63,64); *Chaetophoma* sp. (63); *Cladosporium* sp. (62,63); *Curvaularia* sp. (53,63); *Fusarium* spp. (24,40,53,54,62,63,67); *Penicillium* spp. (40,54,62,63); *Rhizoctonia* sp. (54,63); *Rhizopus* sp. (62,63); *Stemphylium* sp. (63); and *Verticillium* sp. (63).

Internally seedborne bacteria causing losses in seed quality include *Pseudomonas glycinea* Coerper (= *P. syringae* van Hall), causal organism of bacterial blight of soybean (52,54,63,66), and *Bacillus subtilis* Cohn (28,63). Seeds from which these organisms were isolated failed to germinate. Soybean mosaic virus was reported as one causal organism of seed discoloration (54,63), also considered a loss in quality.

In addition to the organisms listed above, five fungi have been reported causing serious problems when associated with soybean seeds: *D. phaseolorum* var. *sojae*; *Peronospora manshurica* (Naoum.)

Syd. ex Gaum. (= *P. sojae* Leh. and Wolf); *Fusarium oxysporum* Schlecht.; *Rhizoctonia solani* Kuehn; and *Pythium debaryanum* Hesse (63).

Pod and stem blight of soybean was first reported in North Carolina in 1920, by Wolf and Lehman. It has now been reported in all major soybean growing areas of the world (48,63). *Diaporthe phaseolorum* var. *sojae*, causal organism of pod and stem blight, is an ascomycete. The imperfect form of the fungus is *Phomopsis sojae* (43,55,63,72). In culture the fungus produces white, fluffy mycelium from which exude yellow-white droplets. Irregular black stromata are formed throughout the culture. Seeds from which the organism is isolated generally fail to germinate (63,71).

D. phaseolorum var. *sojae* is an important cause of low seed quality when internally-borne in soybean seed. Infected seeds generally have a low germination rate, give rise to stunted and diseased seedlings, and produce low quality oil and flour (9,18,22,33,43,51, 55,72,73). Infected seeds and seedlings are also a source of primary inoculum (63). These seeds are often cracked, flattened, smaller in size and volume, lower in density, and covered with grayish-white mycelium. Slightly infected seeds may appear normal, but are still of low quality (33,59,63). Planting of infected seeds may lead to systemic infection of soybean plants (51,63).

The fungus comes into contact with the seeds either through systemic infection of the mature plant (41,51,63), or through cracks and abrasions of the pod walls. These may result from insect damage, mechanical damage, or deterioration of the pod associated with

delayed harvest (4,41,44,75). In an experiment by Athow (4), seeds from pods protected by pollinating bags showed significantly higher germination rates and lower incidence of *D. phaseolorun* var. *sojae*. Athow concluded that systemic infection of soybean plants did not give rise to infected seeds, but technical problems encountered in the experiment may have resulted in invalid data.

Once in contact with the soybean seeds, the fungus colonizes the seed coat, and occasionally the cotyledons and embryo (35,71). Ilyas *et al.* (35) reported that mycelium of *D. phaseolorum* var. *sojae* was found in the hourglass cell layer of the seed coat. They proposed that the starchy matrix of the hourglass cell layer provides a nutrient source for the fungus. Mycelium of the organism spread throughout the seed coat, and pycnidia developed within 72 hours (59). Ellis *et al.* (22) found that most infected seeds were not killed during colonization. Instead, the radicle was killed by the fungus as it emerged from germinating seeds.

Other factors also affect infection of soybean seeds by this fungus. Incidence of internally seedborne *D. phaseolorum* var. *sojae* is higher in seeds from lodged or broken branches in contact with the soil (4,74). Hepperly *et al.* (32) reported that plants infected by soybean mosaic virus were predisposed to seed infection by *D. phaseolorum* var. *sojae*. Dhingra and da Silva (14) found a significant correlation between the presence of weeds around soybean field margins and occurrence of *P. sojae*. They reported that weeds may have contributed to the formation of a microclimate that favored seed infection because of high levels of humidity. Incidence of *P. sojae*

also varied with variety (22), growing region, and fluctuations in temperature and humidity (28).

Downy mildew of soybeans was first reported as a foliar disease by Lehman and Wolf (46). The first specimens were collected near Raleigh, North Carolina, in 1923. In 1942, Johnson and Lefebvre (38) reported soybean seeds encrusted with a whitish material composed of downy mildew oospores.

Peronospora manshurica, the causal organism of downy mildew of soybeans, is an oomycete. The fungus is an obligate parasite, but Dunleavy (17) described a method for inducing germination of oospores in vitro. Oospores from encrusted seeds are globose, 23 to 32 µm in length, and are often surrounded by the collapsed oogonial wall, resulting in the characteristic reticulation on the surface of the oospores (63). Seeds infected by *P. manshurica* are lower in quality, lighter in weight, and have lower germination rates than healthy seeds (16,63).

The fungus overwinters as oospores on seed (45,63). Planting of encrusted seeds can lead to systemic infection of plants, especially when cool soil temperatures cause soybean seeds to germinate slowly (34,39,45,63). Systemically infected plants produce conidia which serve as inoculum for uninfected plants. Seeds from infected plants are generally encrusted with oospores (34,62). Oospore-encrusted seeds also serve as a means of dissemination of the fungus. The first report of downy mildew of soybeans in England was in 1960. The disease organism was probably introduced on infected seed imported from the United States (42).

Some cultivars of soybeans, including Mendota, Kanrich, Kanso, and Midwest, are resistant to all known physiological races of *P. manshurica* (15). Resistance is controlled by a single gene, Rpm (5). The existance of over 25 physiological races of the downy mildew pathogen has been determined using several series of differential cultivars (16,63). The use of resistant varieties is recommended in areas where downy mildew is a recurring problem. Fungicidal seed treatment is effective in increasing germination and emergence, and in reducing the numbers of systemically infected plants (34,54,63).

Fusarium oxysporum, Rhizoctonia solani, and *Pythium debaryanum* are fungi often associated with postemergence damping-off in soybean seedlings, although each organism may also cause other diseases of soybeans (7,8,29,47,63). *F. oxysporum* and *R. solani* have been found internally seedborne (54,63), and *P. debaryanum* is a common soil inhabitant (63).

Fusarium oxysporum is a deuteromycete. The fungus is variable in appearance in culture, but generally produces white, fluffy mycelium. Macroconidia, microconidia, and chlamydospores are produced in vitro (69). When internally seedborne, *F. oxysporum* may cause low germination and postemergence damping-off (63). The fungus penetrates epidermal cells and stomata in the hypocotyl tissues, and invades the cortex of seedlings (29,63).

Rhizoctonia solani is classified in Mycelia Sterilia, and is characterized in culture by robust, closely septate mycelium with

right angle branching. Hyphae are constricted at the points of branching. Sinclair and Shurtleff (63) reported that seeds infected by this fungus either did not germinate or produced seedlings which died shortly after emergence. Boosalis (7) found that fungicidal seed treatments reduced the incidence of damping-off caused by *R. solani*.

Pythium debaryanum is an oomycete. This organism was first reported as the causal organism of a root rot of soybeans in 1926, by Lehman and Wolf (47). Seedlings may become infected by *P. debaryanum* prior to emergence, or by invasion of the hypocotyl region after emergence (8). Infected seedlings characteristically develop lesions on the upper surface of cotyledons, and exhibit greatly enlarged hypocotyl regions (63). This fungus has not been reported to be internally seedborne on soybeans.

CHAPTER III

MATERIALS AND METHODS

Seeds tested in this study were provided by Dr. M. A. Newman from plots in Lake County, Tennessee. The six cultivars used were Bedford, Essex, Forrest, Ring Around-501 (RA-501), York, and Calahan-5 (CA-5). Each cultivar was divided into four plots, each of which received one of four treatments. The treatments were thiabendazole (Mertect 340 F) at the rate of 0.45 l/ha, benomyl (Benlate 50 WP) at 0.58 kg/ha, chlorothalonil (Bravo 6 F) at 2.37 l/ha, and an untreated control. These treatments were applied aerially, from a height of two meters, in 47 liters of water per hectare at early pod set. A second application was made 14 days after the first. Each treatment was replicated three times. After harvest, the seeds were stored at room temperature (24 C ± 3 C) and low humidity, with no moving air currents.

The study was set up in a split-plot design, with 10 replicates of four experiments on seeds from each of the 24 treatment/cultivar combinations. The four experiments were germination, emergence, postemergence damping-off, and isolation of fungi associated with the seeds. Duncan's multiple range test was used to separate means of germination, emergence, and postemergence damping-off by cultivar and treatment.

Germination

There are several accepted methods for testing germination in a seed sample (1,22,52). The method suggested by Newman (50) was used in this experiment because of its constancy and ease of handling large numbers of seeds.

A random sample of 50 seeds from each treatment on each cultivar was used in a single replication, making a total of 1,200 seeds per replication, and an overall total of 12,000 seeds. Each 50-seed sample was positioned between two sheets of germination paper saturated with distilled water. The two sheets were then rolled loosely in a sheet of household wax paper slightly larger than the sheets of germination paper. The roll thus formed was secured loosely with a rubber band. When all of the samples for one replication had been prepared by the above procedure, they were placed in an incubator at 18 C. The rolls of seed samples were misted with distilled water once every 48 hours to maintain a high moisture level around the seeds. After seven days, the samples were unrolled and the radicles measured. All seeds with radicles 3.80 cm or longer were considered to have germinated.

Emergence

Emergence was tested in the greenhouse. A random sample of 50 seeds from each treatment/cultivar combination was used in each replication, and the total number of seeds was the same as for the

germination test. An artificial soil mixture developed by Toler (68) was used as the substrate. The composition of Toler's mixture is given in Table 1. Vermiculite was substituted for the gypsum.

The seeds were planted in standard 10 cm plastic pots sterilized beforehand in a 0.525% solution of sodium hypochlorite. A 150 cm^3 portion of the soil mixture was placed in the bottom of each pot. The seeds were placed on top of this layer, 10 seeds per pot. Finally, a 100 cm^3 portion of the soil mixture was used to cover the seeds. The seedlings were allowed to grow until the first trifoliate leaves developed, then a count was taken of the number of seedlings which emerged, did not emerge, or emerged but damped-off. The first trifoliate leaves were used as indicators to compensate for seasonal variation in temperature and photoperiod in the greenhouse. The duration of each replication ranged from 14 to 21 days.

Isolation

Several techniques have been used to isolate internally seedborne organisms from soybean seeds (1,22,31,65). The method used in this experiment was a synthesis of these techniques.

Seeds for isolation were taken from a random sample of each of the 24 treatment/cultivar combinations. A total of 200 seeds from each combination was used, or 4,800 seeds overall. The seeds were surface sterilized in a freshly-prepared 0.26% solution of sodium hypochlorite in distilled water for one minute, followed by two sterile water rinses. The seeds were blotted on sterile filter

Table 1. Artificial soil mixture of Dr. R. W. Toler

1/2 bushel peat
1/2 bushel perlite
151 grams 6-12-12
76 grams superphosphate
303 grams dolomite
9 grams Sequestrene Fe
303 grams Gypsum

paper to remove excess water, then placed on potato-dextrose agar (PDA) in standard 100 mm diameter petri dishes. The plates were sealed in paper bags, incubated at room temperature, and examined after five days. Organisms growing from seeds were transferred onto fresh PDA plates and maintained in pure culture for identification.

Isolations were also made from seedlings which damped-off after emergence in the greenhouse. The technique used was identical to that used in isolation from seeds, except that pieces of the hypocotyl regions of the damped-off seedlings were used instead of seeds.

CHAPTER IV

RESULTS

Germination

Forrest and Ring Around-501 (RA-501) had significantly higher means of percent germination than the other cultivars, but were not significantly different from each other (Table 2). Calahan-5 (CA-5) had significantly higher germination than Bedford or York, but was not significantly higher than Essex. The mean percent germination for York was significantly lower than that for all other cultivars. Seeds from plants treated with Mertect had a significantly higher mean percent germination than those treated with Bravo or Benlate, but were not significantly different from seeds from the control plants (Table 3). Seeds from control plants were significantly higher than those from plants treated with Benlate in mean percent germination. Seeds from Bravo-treated plants were not significantly different from those from plants treated with Benlate or from control plants.

Emergence

Forrest and Essex had significantly higher emergence than Bedford, CA-5, or York, but were not significantly different from RA-501 (Table 4). Emergence of York was significantly lower than the other cultivars. Seeds from plants treated with Mertect had a

Table 2. Germination of seeds of six soybean cultivars treated with three foliar fungicides, separated by cultivar

Cultivar	Mean*	Grouping
Forrest	82.75	A
RA-501	79.55	A
CA-5	72.45	B
Essex	68.10	BC
Bedford	67.25	C
York	57.70	D

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

Table 3. Effect of selected foliar fungicides on germination of seeds of six soybean cultivars

Treatment	Mean*	Grouping
Mertect	75.43	A
Control	73.33	AB
Bravo	69.73	BC
Benlate	66.70	C

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

Table 4. Emergence of seeds of six soybean cultivars treated with three foliar fungicides, separated by cultivar

Cultivar	Mean*	Grouping
Forrest	88.40	A
Essex	88.35	A
RA-501	87.65	AB
Bedford	85.10	BC
CA-5	84.30	C
York	78.75	D

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

significantly higher mean percent emergence than those from control or Bravo-treated plants, but were not significantly different from seeds from plants treated with Benlate (Table 5). Seeds from Benlate-treated, control, and Bravo-treated plants did not differ significantly in mean percent emergence.

Forrest had a significantly lower mean percent of seedlings damped-off than the other cultivars (Table 6). Forrest, Essex, and CA-5 were significantly different from Bedford, York, and RA-501, but not from each other. There were no significant differences between seeds from plants which received different treatments (Table 7).

Isolation

Fungi isolated from seeds are expressed as percent of total fungi isolated from the seed sample. Fungi isolated from damped-off seedlings are expressed as percent of seedlings damped-off after emergence in the greenhouse. There were no significant correlations between the incidence of fungi isolated and germination, emergence, postemergence damping-off, cultivar, treatment, or replication.

Diaporthe phaseolorum var. *sojae* was found internally seedborne in 5.58% of the seeds. The fungus was identified in vitro through the cooperation of Dr. J. W. Hilty and P. J. Goodfellow, Professor and Graduate Research Assistant, respectively, in the Department of Entomology and Plant Pathology at The University of Tennessee. The fungus was induced to produce pycnidia in culture following methods described by Sinclair and Shurtleff (63).

Table 5. Effect of selected foliar fungicides on emergence of seeds of six soybean cultivars

Treatment	Mean*	Grouping
Mertect	87.57	A
Benlate	85.30	AB
Control	84.73	B
Bravo	84.10	B

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

Table 6. Postemergence damping-off of seeds of six soybean cultivars treated with three foliar fungicides, separated by cultivar

Cultivar	Mean*	Grouping
Forrest	1.30	A
Essex	1.50	AB
CA-5	1.55	AB
Bedford	2.20	B
York	2.30	B
RA-501	2.35	B

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

Table 7. Effect of selected foliar fungicides on postemergence damping-off of seeds of six soybean cultivars

Treatment	Mean*	Grouping
Control	1.60	A
Mertect	1.80	A
Bravo	2.00	A
Benlate	2.07	A

*Values are the means of 10 replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test (P = .05).

Peronospora manshurica occurred as a characteristic encrustation on 2.83% of the seeds. The fungus was identified from the descriptions in Sinclair and Shurtleff (63) of both macro- and microscopic characters.

Fusarium oxysporum was isolated from 8.88% of the damped-off seedlings. *Rhizoctonia solani* and *Pythium debaryanum* were found 0.54% and 0.63% of the time, respectively. Isolations from hypocotyls of other damped-off seedlings in the sample yielded no fungi, or were contaminated by bacteria.

The mean percent of total fungi in the seed sample was 19.58%. In addition to those above, the following fungi were isolated: *Alternaria tenuis* Nees; *Aspergillus* spp.; *Chaetomium erectum* Skolko and Groves; *Chaetophoma* sp.; *Cladosporium* sp.; *Curvularia lunata* (Wakker) Boedijn; *Fusarium solani* (Mart.) Sacc.; *Penicillium* spp.; *Rhizopus nigricans* Ehr.; *Stemphylium* sp.; and *Verticillium* sp. References used in the identification of these fungi were Ellis (25,26) and Toussoun and Nelson (69). Three fungi were not identified, although one isolate was tentatively identified as a species of *Colletotrichum* by Dr. E. S. Luttrell of The University of Georgia.

CHAPTER V

DISCUSSION

Germination

Application of foliar fungicides had no apparent effect on seed quality in this study. Seeds from plants treated with Mertect 340 F (0.45 l/ha), Benlate 50 WP (0.58 kg/ha), or Bravo 6 F (2.37 l/ha) showed no significant increase in germination or decrease in occurrence of internally-borne fungi over seeds from control plants. These results differ with most reports on previous experiments of this kind. Mertect (6,57,58,62), Benlate (6,18,21,24,27,36,57,58, 60,62,70), and Bravo (6,57,58,62) were reported effective in increasing percent germination in vitro, and in reducing the incidence of *Diaporthe phaseolorum* var. *sojae* and other internally seedborne fungi when applied as foliar sprays. Reports that Mertect (27) or Bravo (27,36) were not effective in improving seed quality were limited, and no such reports were found concerning Benlate. Differences in rates or methods of application could influence results. The fungicides in this study were applied aerially, in 47 liters of water per hectare, from a height of two meters. Aerial application is more subject to drift or uneven coverage of plots than application from a hand sprayer or tractor boom.

Chamberlain and Gray (10) stated that seeds with a germination rate below 80% are unacceptable, and seeds germinating below the 60%

level should be rejected. The germination mean in the present study was 71.30%. By these criteria, only Forrest and, marginally, Ring Around-501 (RA-501), had acceptable germination rates. Calahan-5 (CA-5), Essex, and Bedford were unacceptable, and York should have been rejected. The low germination rates demonstrated by seeds in this study cannot be explained strictly in terms of seed response to internally seedborne pathogens. The majority of seeds which failed to germinate exhibited no fungal or bacterial growth. These seeds either imbibed with no elongation of the radicle, or produced a radicle of insufficient length to be considered as germinated. A small percentage of seeds failed even to imbibe. Nor was this lack of vigor attributable to aging or storage conditions of the seeds. The seeds were stored at room temperature (24 C ± 3 C) and low humidity, with no moving air currents. Kennedy (40) reported that the incidence of some seedborne fungi actually decreased under conditions similar to these.

Emergence

At 85.43%, the emergence mean was significantly higher than the mean for germination, which was 71.30%. This difference could have resulted from the temperature at which each test was performed. The emergence test was performed at ambient temperature in the greenhouse, but the germination test was run in an incubator at 18 C. Lehman (45) and Sinclair and Shurtleff (63) reported that seeds which germinate in a cool environment are more susceptible to invasion

by seedborne pathogens. Seeds in the emergence test germinated more quickly, were less susceptible, and produced a higher percentage of healthy seedlings. Another possible explanation is that seeds which had produced a radicle less than 3.80 cm long in a replication of the germination test were not considered as germinated. In the greenhouse, these seeds would have had from two to three times longer to develop, and might have produced plants which, even though less vigorous, would have emerged and been included in the count.

Isolation

Analysis of the correlation performed in this study showed that the fungicide treatments had no significant effect on the incidence of internally seedborne fungi. The percent occurrence of *D. phaseolorum* var. *sojae* or *Peronospora manshurica* in seeds from treated plants was not significantly different from that in seeds from the controls. The effect of treatment on fungi isolated from damped-off seedlings was not significant either. *Fusarium oxysporum* and *Rhizoctonia solani* were isolated from seeds and from hypocotyls of seedlings, but *Pythium debaryanum* was isolated only from seedlings which had damped-off after emergence. *P. debaryanum* has not been reported internally seedborne on soybeans. It is a common soil inhabitant (63), but occurrence of the fungus in the artificial soil mixture is unlikely because the peat used in the mixture was autoclaved before being added to the other constituents. A possible explanation for the presence of this fungus is that it was borne

externally on the soybean seeds used in the emergence test. Debris from pods and petioles mixed in with the seeds at harvest may have served as an inoculum source.

A portion of the seeds in the sample showed staining around the hilum characteristic of purple seed stain caused by *Cercospora kikuchii*. This organism was never isolated even though several techniques were used (49,63). The staining may have been caused by soybean mosaic virus instead of *C. kikuchii*. Possibly, *C. kikuchii* was not detected because of other fungi, such as *Fusarium* or *Alternaria* spp., which also occurred on the seed. These organisms grow much faster in culture, and may have masked the presence of *C. kikuchii*.

Conclusions

Due to variability in the results of this study, only guarded conclusions may be advanced. The data indicate that Mertect, Benlate, and Bravo had no significant effects on seed quality when applied aerially as foliar sprays. York cultivar had significantly lower means of percent germination and emergence than other cultivars. Forrest had the highest mean percent of germination and emergence, and the lowest mean percent of seedlings damped-off after emergence, but the differences were not significant from all other cultivars at the .05 level of probability.

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