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EFFECT OF GENOTYPIC VARIATION AND DELAYED HARVEST UPON SEED QUALITY IN PHASEOLUS VULGARIS L . UNDER CONDITIONS OF INTERNAL SEED-BORNE FUNGAL INFECTION.

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EFFECTS OF GENOTYPIC VARIATION AND DELAYED HARVEST UPON SEED QUALITY IN *PHASEOLUS VULGARIS* L. UNDER CONDITIONS OF INTERNAL SEED-BORNE FUNGAL INFECTION

By

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ABSTRACT

EFFECTS OF GENOTYPIC VARIATION AND DELAYED HARVEST UPON SEED QUALITY IN PHASEOLUS VULGARIS L. UNDER CONDITIONS OF INTERNAL SEED-BORNE FUNGAL INFECTION

By

Krishna Prasad Sharma

Potential genetic variability and effect of delayed harvest after normal maturity for: the extent of external and internal seed infection by fungi, in vitro seed germination, field emergence, specific fungus-genotype interactions, and genetics of resistance to internal seed-borne fungi in dry bean (Phaseolus vulgaris L) were studied in the laboratory, green-house, and in the field from 1977 to 1979. Forty-two bean genotypes were used in preliminary screening for resistance to internal and external seed-borne fungi. Non-surface sterilized and surface-sterilized seeds were incubated in SPDA plates for 5-6 days under normal light and temperature to study external and internal seed infection, respectively. Seeds were surface-sterilized for one minute in 1:1 NaOCl solution (2.6% a.i.) and blotted dry.

Crosses between San-Fernando (resistant) and Tuscola (susceptible) cultivars were made in the green-house. F_1 and seven other selected genotypes including the parents were planted in the field. Plots were sprayed with a mold spore suspension at physiological maturity. Seeds were harvested at three different times: normal maturity, two weeks after, and three weeks after normal maturity. Four hundred surface-sterilized seeds/genotype/harvest date and 200 seeds/plant for fifteen F_1 plants harvested two weeks after normal

maturity were used for internal seed infection study. *In vitro* seed germination and field emergence tests were made by using 400 non-surface-sterilized seeds/genotype/harvest date.

No genotypic variation for external seed infection and a large genotypic variation for internal seed infection by fungi were observed. Harvest date X genotype interaction was highly statistically significant. San-Fernando, Nep-2, Turrialba #1, and Ex-Rico-23 showed negative effects while Tuscola, BTS, and Seafarer showed positive effects of delayed harvest. Eight fungi: *Alternaria*, *Rhizoctonia*, *Fusarium*, *Penicillium*, *Cladosporium*, *Epiccoccum*, *Chaetomium*, and *Rhizopus* were isolated from surface-sterilized seed; the first two fungi showed specific fungus X genotype interactions. Many pairs of genes with additive effects or showing partial dominance over susceptibility are postulated to confer resistance. Significant genotypic variation for *in vitro* seed germination and field emergence was observed. Harvest delay did not show a significant reduction in *in vitro* seed germination of the resistant parent. Black and thick seed coated genotypes showed superiority to white and thin seed coated genotypes for field emergence. *In vitro* seed germination overpredicted the field emergence of Nep-2.

To

my Parents

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INTRODUCTION

After ripening, mold can be a major problem in the successful production of seed of dry beans (Phaseolus vulgaris L.) both in tropical and temperate zones of the world. The molded plants are usually coated with a fine layer of blackish dust which represents infected pods (Figure 10), leaves, and plant as a whole. The fungi causing mold may be external and/or internal seed-borne. The later type is more important from seed quality standpoints. The fungi get pulverized during threshing and the seeds are surface contaminated with viable micro-organisms. They alter the bio-chemical composition of the stored bean reducing commercial value (140). These fungi have a tremendous effect reducing seed germination and field emergence. High temperature (around 85°F), high relative humidity, and a delay in harvest past maturity provide congenial conditions for mold invasion. The fungi which are internally seed-borne have been reported to reduce dry bean production as much as 50 percent and complete crop failure is not unusual (34) in some individual cases.

The yield reduction in dry beans and soybeans in the tropics by internally seed-borne fungi has been reported by many workers. The seed is one of the most basic elements to any crop production program and without high seed germination and seedling emergence in the field there is no efficient crop production. One of the major problems facing increased dry bean production in the tropics is a reliable

source of high quality disease free seeds for planting (3). The same is true in the temperate regions when harvest time is accompanied by rainfall which causes a delay in harvesting. Seed deterioration is highly associated with the percentage of internally-borne fungi. However, colored-seeded genotypes have been reported to do better under such conditions often out-yielding the white-seeded counterpart, color preferences have caused complications in bean production (4,5,10,26, 28,55,92,115,124). The fungus Aspergillus flavus, which causes molding, produces the toxin "aflatoxin" which has potent carcinogenic properties (140). The toxin also causes death in poultry and abortion in higher animals.

Michigan, a leading dry bean producing state with 34 percent of the national total, produced 6,440,000 hundred weight (cwt) clean beans, 14 percent larger than in 1977 of which 90 percent were navy beans (88). Growers increased the planted acreage over 1977 only by 4 percent. Low yields in 1977 were due in part to crop abandonment after rainfalls during time of harvest. The crop was abandoned because of the high degree of mold invasion. Seed quality may be completely deteriorated if the storage conditions are favorable for the mold which is already present in the seeds, to develop.

The objectives of this study were: (1) to determine the possible existence of genetic variation for incidence of internal seed infection by fungi; (2) to identify the fungi involved and determine possible specific genotype-fungus interactions (3) to determine the effect of harvest date on seed quality, and (4) to study the genetics of resistence for incidence of mold invasion.

LITERATURE REVIEW

The importance of seed-borne fungi in different crops was recognized when harvested products (seeds) stored under high temperature and moisture conditions developed mold which decreased seed quality and viability. Infected seed exhibited poor field emergence and stand when planted. This fact prompted researchers; 1) to study the genera and species of fungi involved, 2) to study proper storage conditions and; 3) to improve seed quality and viability.

There are two types of seed-borne fungi; 1) external seed-borne fungi and 2) internal seed-borne. Internal seed-borne fungi constitute the major problem because fungicidal treatments are not effective. The original seed quality problems can be attributed to two major stages; a) Seed production and b) Seed storage (3). Problems encountered in the production of high quality seed include; seed infection in the field by micro-organisms, damage to pods and seeds from feeding insects; unfavorable growing conditions for the crop including attack from diseases, insects, and weeds, and unfavorable climatic conditions (rain and high temperature) at time of harvest. Seed storage problems are mainly due to conditions of high temperature and relative humidity during storage of seed already contaminated with viable micro-organisms.

Poor seed quality is generally reflected by low laboratory seed germination and field emergence (61). Low moisture content is a key

factor in maintaining seed viability (148).

I. Dry Beans (Phaseolus vulgaris L.)

Numerous fungi have been reported to be borne internally in seeds of bean;(Acrostalomus sp (24,44); Alternaria sp (24,34,41,44, 83,127); Aspergillus sp (18,24,44,84); Botrytis sp (24,44); Cladosporium sp (24,41,44); Colletotrichum sp (24,34,44); Fusarium sp (24,34, 39,41,43,44,64,102,127); Isariopsis sp (24,44); Macrophoma sp (24); Macrophomina sp (34,41,44,102); Monilia sp (24,41,44); Nigrospora sp (41); Penicillium sp (24,41,46); Pestalotia sp (24,41); Peyronellaea sp (24); Phomopsis sp (24,34,39,41,44,64,114); Phoma sp (34,41); Rhizoctonia sp (24,28,29,34,41,44,46,79,102,112,127); Rhizopus sp (24); Sclerotinia sp (15,24,44); and Trichothecium sp (34).

Incidence of internal seed-borne fungi is negatively correlated with seed quality, seed germination, emergence and seedling vigor in Phaseolus vulgaris (24,29,34,39,43,44,46,123,163). Of seed that contained fungi, 71% did not germinate (24). Dingra in Brazil (123) concluded that dry and snap bean seed grown during the rainy season and harvested at normal maturity had very poor germination. Seed infected internally with fungi germinated only 68%.

There are high correlations between moisture content, relative humidity, mold count, and duration of storage (13,18,120). Lopez et al. (84) in a study of moisture content, number and type of fungi present after three months storage at 18.5-22% moisture, found no positive correlations between degree or rate of moisture absorption and fungal infection. Defective seedlings such as "baldhead" and "snakehead" eventually resulted from invasion of the seeds by storage fungi (18).

Invasion occurred through the hilum and micropyle as well as through cracks in the seed coat; the embryo root, stem, and growing point appeared to be attacked in preference to the cotyledon (18). Preliminary pathogenicity tests indicate that the fungi could penetrate through an uninjured pod wall and infect developing seed (34). He also reported that the fungi grew out through the hilum and micropyle or through the cracks in the seed coat but never through the unbroken seed coat (18), and concluded that if the coats of the seeds invaded by storage fungi were punctured, the fungi grew out through these punctures by increasing the mold invasion.

Discoloration causes by one pathogen is indistinguishable from that caused by others (34), and such discoloration not only reduces seed quality but also reduces the commercial value of the seed for human consumption.

Harvesting at proper maturity (113,132,133) increases seed quality in dry beans. Prolonged rain after full pod set predisposes pods and seeds to fungal invasion by allowing prolonged periods of high humidity and pod surface wetness (34,39). However, occurrence of fungi infecting dry bean seed varies from location to location and cultivar to cultivar (34).

One problem in the production of high quality dry bean seed is pod contact with soil (23,41). Percent internal seed-borne fungi among cultivars with pods in contact with soil ranged from 64-92% as compared to 3-30% with pods not in contact with soil (41). The mean percent germination and field emergence of seeds from pods not in contact with soil was significantly higher than that of seed from pods in contact with soil. Seed treatment had no effect on germination

of high quality seed but greatly improved that of poor quality seed (22,45). Plants having many pods in contact with soil are therefore considered poor architectural types, and such plants are also prone to seed-borne diseases.

Varietal differences in degree of fungal invasion, seed germination and field emergence have been reported for the bean. Varietal differences for Rhizoctonia solani resistance were observed by Prasad et al. (112); resistance was highly heritable and associated with colored seed (29). Snap bean cultivars (Phaseolus vulgaris L.) with colored seeds produced stronger and more vigorous seedlings than those with white seed (68). Cultivars with colored seed appeared to adapt much better to adverse conditions than white seeded cultivars. Deakin (28) reported that the mean percentage of emergence showed no significant differences among genotypes although some variability existed. Lines (genotypes) by color interactions were also nonsignificant. Emergence of color seeded lines was almost always superior to that of their white seeded counterpart; such superiority was thought due to resistance to Rhizoctonia solani.

Cesar, et al. (18) also found varietal differences in percent seed germination and number of abnormal seedlings among three bean genotypes; Amarillo, Jamapa, and Bayomex. The superiority of Jamapa was thought due to sound seed coats with no detectable breaks or cracks. Much of the seed of the Bayomex and Amarilo 153 varieties had obvious breaks and cracks in the coats and these cracks probably allowed ready invasion of fungi. Hard seed coats appear to inhibit invasion by micro-organisms (78). Seed treatment has little effect on internal seed-borne fungi in dry bean, particularly when conditions

are optimal for disease development (79).

II. Soybean (Glycine max)

Poor soybean seed quality in the northern USA is primarily the result of infection with a species of Phomopsis (Diaporthe phaseolorum var sojae) and Diaporthe phaseolorum var caulivora (126). These fungi can infect seed still contained in the pod, but cause the greatest damage through latent seed infection which leads to seedling rot during germination. Seed germination is inversely related to percentage seed infection. Infection remains latent until pods begin to mature during wet weather. Poor seed quality is also a major problem of soybean production in tropical conditions (138). High temperature and humidity at planting time (101) and rain at or during the maturity period (33,101,145) were favorable for seedling rot and pod diseases leading to seed damage.

Many fungi have been reported to be internally seed-borne in soybean; Alternaria sp (3,40,56,73,89,98,147,163,159); Arthinum sp (3); Asperigillus sp (3,32,38,56,73,74,91,98,145,149,159); Cercospora sp (3,33,56,57,70,71,74,98,140,156,158,159); Cephalosporium sp (3,74); Chaetonium sp (3,98,159); Chaetophoma sp (3,74); Chanephora sp (3); Colletotrichum sp (3,40,74,163); Corynespora cassilicola (3); Fusarium sp (3,40,56,57,73,74,89,98,121,163,159); Gliocladium sp (3,74); Glomerella sp (3,74); Helminthosporium sp (3); Lasidiplodia sp (3,74); Leptosphaerolina sp (3,74); Macrophoma sp (3,74); Macrophomina sp (2,40,74,163); Myrothecium sp (74); Nodulosporium sp (3); Penicillium sp (3,56,59,73,74,98,159); Pestalotia sp (3,74,98,159); Phomopsis sp (3,6,16,19,27,36,40,50,56,57,75,74,99,106,140,151,152,163,162); Phoma sp (3,74); Populina sp (3); Rhizoctonia sp (3,57,74); Rhizopus sp

(3,74,98,159); Sclerotinia sp (98,99,159); Sclerotium sp (3,74); Syncephalastium sp (3); Thielaviopsis basicola (98,159); Trichocladium sp (3); Trichoderma sp (3,74).

The majority of fungi isolated from soybean seeds appear to be saprophytes which have no noticeable effect on seed germination (3). It was concluded that, of thirty-five fungi tested, only nineteen significantly reduced germination in vitro and the most pathogenic were Nodulosporium sp, Sclerotium sp, Leptosphaerulina sp, Phomopsis sp, Lasidiplodia sp, Aspergillus sp, Colletotrichum sp, Macrophoma sp, Macrophomina sp, and Cephalosporium sp. Fourteen fungi significantly reduced emergence in sand tests. Pod and stem blight caused by Phomopsis sp are among the principle diseases associated with reduced seed germination, and viability and seed quality deterioration in many soybean production areas (6,19,27,36,75,106,140,151,152); however, the fungus did not reduce in vitro germination or field emergence when compared to Sclerotinia sp (99).

Wilcox (157) and Ellis (40) reported that increases in percentage of soybean seed infected by internally seed-borne fungi such as Phomopsis, Fusarium, and Alternaria sp were accompanied by increases in percentage seed germination and decreases in field emergence. Aspergillus sp, a major problem in stored soybean has a significant negative correlation with seed viability, germinability, and also causes seedling blight (32,38,73,91,145,149) particularly under conditions of high temperature and moisture (90). Many other workers (2,93,96,97,117,134) have reported that micro-organisms infecting seed reduce seed quality and often cause low germinability and seed deterioration in soybeans.

Cercospora sp causes purple stain in soybean seed and is associated with poor seed quality (73,138,156). This diesease may reduce yield considerably, and decrease quality and market value of the product. Incidence of the purple stain disease is most severe when the period of seed maturity occurs during wet weather conditinos (73,140). Resistance to purple stain is highly heritable (H=.91) (158).

Delayed harvest did not significantly affect soybean yield but reduced percentage seedling emergence (101) when harvested seed was planted. Delay in harvest also significantly reduced emergence (104, 156) and the average sand emergence percentages were 95, 88, and 74 percent and field emergence percentages were 90, 77, 57 percent for the non-delayed, two weeks-delayed and four weeks-delayed harvest dates respectively ((132)) It was concluded that incidence of internal seed-borne fungi increased significantly when harvest was delayed beyond normal maturity; and that the incidence was negatively correlated with germination and field emergence (40,104,157) However, length of storage is the major factor influencing seed germination while delayed harvest had less but a more variable effect (154).

Plants maturing under dry conditions had less fungal infection so that harvest delays and fungicidal treatments had no effect on incidence of fungal seed infection and germination (153). However, delayed harvest of plants maturing under wet conditions resulted in seed viability reductions of 25% with significant cultivar X harvest time interaction. Alexander et al. (1) in experiments conducted for four years found that, a relatively low percentage of soybeans was infected and germination was excellent at normal harvest. At each succeeding harvest, percentage infection increased and germination

decreased. At the last harvest most beans were infected and only few germinated normally.

The presence of Phomopsis sojae, Fusarium semitectum and Colletotrichum dematium f. truncata in soybean plantings was significantly correlated with weed development, suggesting that weeds may serve as alternate hosts or provide a microclimate of prolonged high humidity favoring seed infection (33). However, the occurrences of Macrophomina phaseolina and Cercospora kukuchii were not affected by weed development.

Paschal et al. (154) found consistent differences in germinability among soybean lines with some small seeded lines from southeast Asia maintaining more than 50% germination after eight months of storage under ambient environmental conditions. Seed size was negatively correlated with field emergence and positively correlated with incidence of internal fungi (104). Smaller-seeded genotypes had higher emergence percentage and less internally seed-borne fungi.

Seed lots of twelve soybean cultivars harvested from different growing locations over a three year period showed significant differences in percentage germination between years, and in occurrence of seed-borne micro-organisms between four locations and years (38,56). The occurrence of seed-borne micro-organisms was influenced more by growing location than by different planting dates, harvesting dates (145) or method of harvest (98,99). Tedia (145) found that year to year fluctuations in incidence of seed-borne micro-organisms in soybean and the associated decline in seed vigor prior to physiological maturity were due to high temperatures during seed maturation. The differences in occurrence of micro-organisms between growing locations

was expected because of differences in rainfall near harvest (38).

It has been concluded that in the absence of soybean cultivars resistant to pod and seed infection, seed should be harvested as soon as possible after normal maturity (104,135,156).

There is a great variation in seed quality among soybean cultivars (38,50,104,138). Percentage seed infection and *in vitro* germination ranged from 0-100% (104). Percentage germination and total fungi differed significantly among the seed lots between regions for Wayne but not for Amsoy cultivars (146). Cultivar interactions were noted in germination incidence of total seed-borne fungi (56).

Soybean cultivars Hardee and PI 205 912 inoculated with *Phomopsis* spore suspensions showed no significant difference in percentage *Phomopsis* infection of harvested seed. However, seeds of PI 205 912 which were infected by *Phomopsis* had a significantly higher *in vitro* germination percentage than infected seeds of Hardee, suggesting that PI 205 912 may possess tolerance against seed or seedling decay (50,162).

III. Pea (*Pisum sativum*)

Storage fungi reduce germination percentage of pea (*Pisum sativum*) seed (53). A genetic study of tolerance to *Aphanomyces* root rot found resistance to be associated with the presence of pigmentation in flowers and seeds (85). In a study on the importance of testa color in resistance to *Pythium ultimum*, Stasz (142) reported that resistance was found only in plants where seed possessed color testa other than white.

Alternaria is reported to cause a seed spot in pea. With

increasing temperature, moisture content and time, percentage of internally seed-borne micro-organisms such as Asperigillus sp increases (35). Increased populations of certain pathogens could then increase infection of the host (12). The association of production disease has also been reported (12).

IV. Pigeon Pea (Cajanus cajan)

Internal seed-borne fungi play a major role in reducing the quality of pigeon pea (Cajanus cajan) seed (42,47). Ellis et al. (48) reported that differences in pigeon pea seed quality were due to the temperature and humidity of the growing location. Low percentage seed germination was associated with poor physical appearance and higher incidence of internally seed-borne fungi. Presence of Phomopsis, Fusarium and Lasidiplodia was negatively correlated with germination and field emergence (47,48). The incidence of Alternaria was not significantly correlated with in vitro germination or with field emergence indicating that Alternaria does not adversely affect seed germination (47); also, incidence of Aspergillus was not correlated with field emergence (48). Penicillium and Rhizopus sp were also isolated from pigeon pea seed.

Seed infection increased with time after normal maturity. When seeds were harvested at normal maturity, populations of internal seed-borne fungi were very low and emergence in the field was greater than 90% (47). This shows the importance of timely harvest. Breeding for uniform maturation is suggested for pigeon pea as pods do not mature simultaneously in present cultivars.

Different seed lots (150) of pigeon pea were tested for

seed-borne fungi and it was found that 53.7% of seeds in original unselected bulk were infected as compared to 41.7% infection in brown seeded bulk, and 78.7% in light seeded bulk progenies. Brown colored seed were healthier and yielded 14.4% greater. Light brown colored seed were small, yielded less and were more heavily infested with fungi.

V. Cow Pea (Vigna unguiculata)

Ellis et al (65) harvested cow pea (Vigna unguiculata) seed at normal maturity and at one, two, or three week thereafter. Alternaria, Cladosporium, Fusarium, Lasidiplodia, and Phomopsis were isolated from surface dis-infected seeds. Incidence of internal seed-borne fungi increased and the percent seed germination in vitro and field emergence decreased with each delay in harvest. Fungicidal sprays did not prevent the decline in seed quality. When harvested at normal maturity, plants yielded high quality seed. Crops harvested when 75-80% of the pods were dry (103) yielded well and harvested seed was of good quality. Percent of high (103,115) quality seed was least when pods were allowed to dry completely before harvest.

VI. Miscellaneous (other legumes)

Bennett et al. (14) reported that good quality seeds could be obtained by harvesting seeds of rough pea (Lathyrus hirsutum) about one week earlier than usual and drying them. Delay in harvest accompanied by high temperature and humidity increased mold invasion.

Seeds of black gram (Phaseolus mungo), lentil (Lens esculenta), and moth bean (Phaseolus acotinifolius) were found infected with a variety of fungi (144). Seed-borne fungi isolated by the blotter

method were species of Alternaria, Cladosporium, Macrophomina, and Fusarium; artificial innoculations with these fungi reduced seed vigor and viability. While seed treatment with fungicides did not completely eliminate the seed-borne fungi, it did reduce number of fungi. Singh et al. (136) isolated the following fungi from surface-sterilized black gram seed; Aspergillus, Fusarium, Phoma, (which had no effect on germination) Curvularia, which affected both seed germination and seedling vigor, and Penicillium, which resulted in poor root development.

Singh et al. (137) reported association of the following fungi with seed of chick pea (Cicer arietinum); Cladosporium sp (19%), Curvularia sp (52%), Fusarium sp (19%), Penicillium sp (18%), Pleospora sp (41%), Rhizopus sp (5%), and Trichothecium sp (10%). All fungi were pathogenic on seeds and seedlings except Curvularia, Penicillium, and Rhizopus. Pleospora caused severe seed rot and dark root lesions which later caused seedling death. Trichothecium decreased seedling vigor. Cladosporium caused a stubby root condition and poor root growth vigor. Fungicide treatments only increased percentage germination from 2-20%.

CHAPTER I

PRELIMINARY STUDY ON GENOTYPIC VARIATION FOR EXTERNAL AND INTERNAL SEED CONTAMINATION BY FUNGI IN DRY BEANS (*Phaseolus vulgaris* L)

Materials and Methods

Sample Collection

Heavy rains and hail in September 1977 damaged a large portion of the Michigan dry bean crop. The wet weather was associated with high temperatures and resulted in considerable molding of pods and seeds on crop plants still standing in the field. Molded pods of forty-two genotypes obtained from an international Bean Yield Nursery grown at the Saginaw, Michigan Bean and Beet Research Farm were collected to study the extent of internal and external seed contamination by fungi. Pods were hand-threshed and stored at room temperature for about 60 days until tested.

Media Preparation

Thirty-nine grams of commercial Difco Potato Dextrose Agar (PDA) were added to one litre of distilled water. The mixture was put in a steamer at 100°C until the PDA was completely dissolved. The solution was autoclaved for twenty minutes at 132.2°C and 1.1 kg/cm^2 steam pressure. One ml of a 200 ppm streptamycin sulfate solution was added to each 100 ml of sterilized PDA. Streptomycin potato dextrose

agar (SPDA) was poured in sterile 100 x 15 mm Dispo Petri Dishes and incubated for thirty-six hours; plates showing growth of contaminants at this time were discarded.

Assay Technique

Twenty non-surface sterilized seeds of each genotype were placed in SPDA plates (10 seeds per plate) to determine incidence of external seed contamination by fungi. Seeds were incubated at 24 ± 1°C in the laboratory, and incidence of fungi growing from the seed was recorded after 5-6 days.

To determine incidence of internal seed contamination twenty uncracked seeds from each genotype were randomly taken and surface-sterilized by soaking for two minutes in 1:1 aqueous bleach solution (2.6% NaOCl). The seeds were immediately dried on paper towelling and transferred individually to SPDA plates. Instruments used in handling seeds were dipped in 95% ethyl alcohol and thoroughly flamed between individual seeds. Seed transfers and all culture work were performed in a standard transfer chamber. Seeds were incubated at 24 ± 1°C in the laboratory and number of infected seeds were recorded after 5-6 days.

The same assays were repeated in two replications for thirteen promising and one susceptible genotype; fungal counts were averaged and are expressed in percentage of infected seeds.

Results

The results show no significant genotypic variation in degree of external seed contamination by fungi. Contamination ranged from 80-100% as shown in Table 1. Of forty-two genotypes tested,

Table 1. Percentage external infection of dry bean (*Phaseolus vulgaris* L) seeds by fungi

Genotype	Seed coat color	Percent infection (20 seeds)
Campbell Soup 105	white	80
Porrille Sintetico	black	85
Campbell Soup 109	white	90
PI 313 868	black	90
Linea 29	black	90
Ex-Rico-23	white	90
MSU Line 20489	white	90
Campbell Soup 106	white	90
Campbell Soup 107	white	90
Campbell Soup 103	white	90
Campbell Soup 101	white	90
ICA Huasano	black	95
Jalpatagua 72	black	95
PI 310 333	black	95
Jamapa	black	95
PI 169 299	white	95
Mexico 12-1	dark brown	95
C 63 S 630-B	beige	95
Campbell Soup 104	white	95
25-M-($3F_5$)	black	100
I-968	black	100
21-M-($3F_5$)	black	100

Table 1. (Continued)

Genotype	Seed coat color	Percent infection (20 seeds)
Collection 168 N	black	100
Pecho Amarillo	black	100
M-Gearais	black	100
PI 310 740	black	100
San-Pedero Pinula 72	black	100
R-345-LRK OZ	pink	100
R.K. 7690	red	100
Nep-2	white	100
PI 284 703	light brown	100
Lamaniere	purpure mottled	100
Mshuizaico	red	100
Brasil 2	brown	100
Redkloud	pink	100
Tuscola	white	100
Sanilac	white	100
Campbell Soup 110	white	100
Campbell Soup 102	white	100
Atlas R-9	white	100
PI 196 936	white	100
San-Fernando (S-182 N)	balck	100

twenty-three genotypes had 100% contamination. The remaining genotypes were contaminated in the range of 90% and above, although two showed less than 90% contamination (fig. 1). Of twenty-three genotypes with 100% seed contamination, fifteen were color-seeded. Seed coat color apparently had little or no effect upon external seed contamination. The frequency distribution of genotypes and percent contamination is shown in Figure 1.

All the genotypes were then tested for internal seed infection by fungi. Internal seed contamination ranged from 0-90% (Table 2) and there was considerable genotypic variation. San-Fernando, a black seeded genotype, was entirely free of internal seed contamination. Thirteen genotypes ranged from 15-30% contamination of which nine were colored seeded and four white seeded.

In general, colored seeded genotypes showed overall superiority to the white seeded genotypes. Some of the white seeded lines, such as Ex-Rico-23 and Campbell Soup 109 did as well as the other colored seeded lines. Black seed coat color showed general superiority to other seed coat colors. However, one black seeded genotype, 21-M-($3F_5$), showed 70% internal seed contamination.

Of the forty-two genotypes tested for internal seed contamination, fourteen were selected for further study; thirteen showed very low and one showed very high infection levels. The percentage internal seed contamination ranged from 1-75% (Table 3) which suggests large genotypic variation to internal seed infection. Of the fourteen genotypes tested three were contaminated in the range of 0-15% (all color seeded); five genotypes were in the range of 16-30%, of which four were colored and one white. One genotype, white-seeded Sanilac,

showed 75% infection. San-Fernando again showed only 1% internal seed contamination even though external seed contamination was 100%.

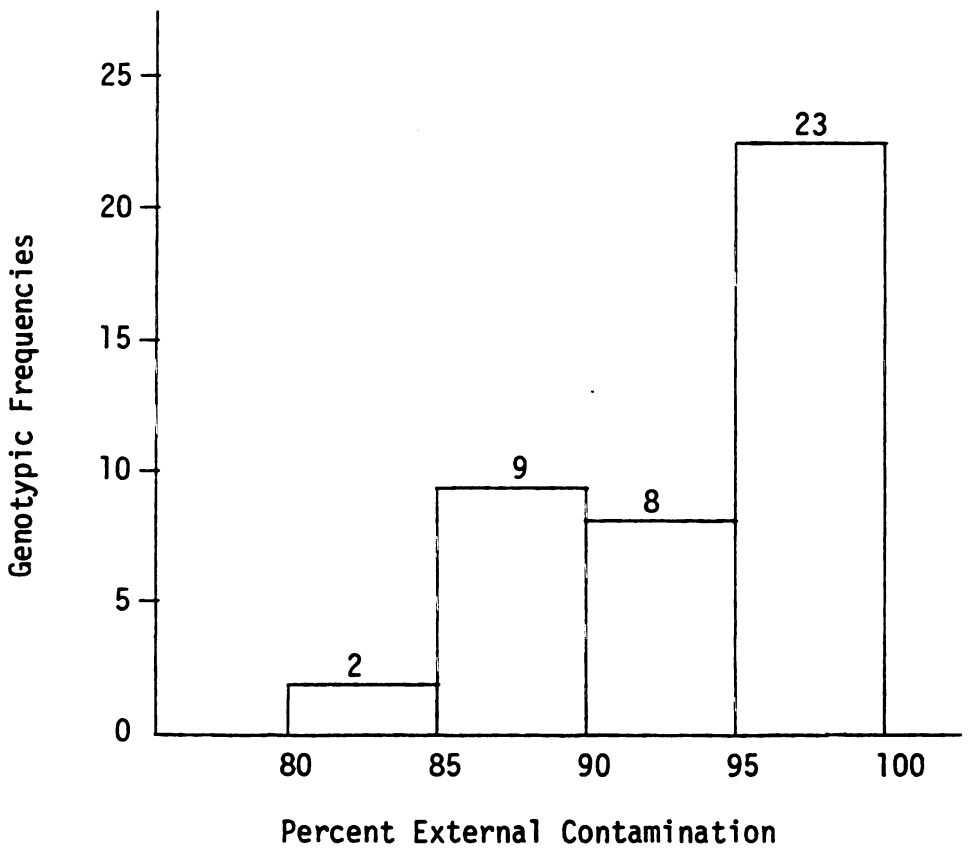


Figure 1. Degree of external seed contamination by fungi in forty-two bean genotypes

Genotypes*	
Colored	White
A = 100%	0%
B = 76.92%	23.08%
C = 75%	25%
D = 54.54%	45.46%
E = 20%	80%
F = 50%	50%

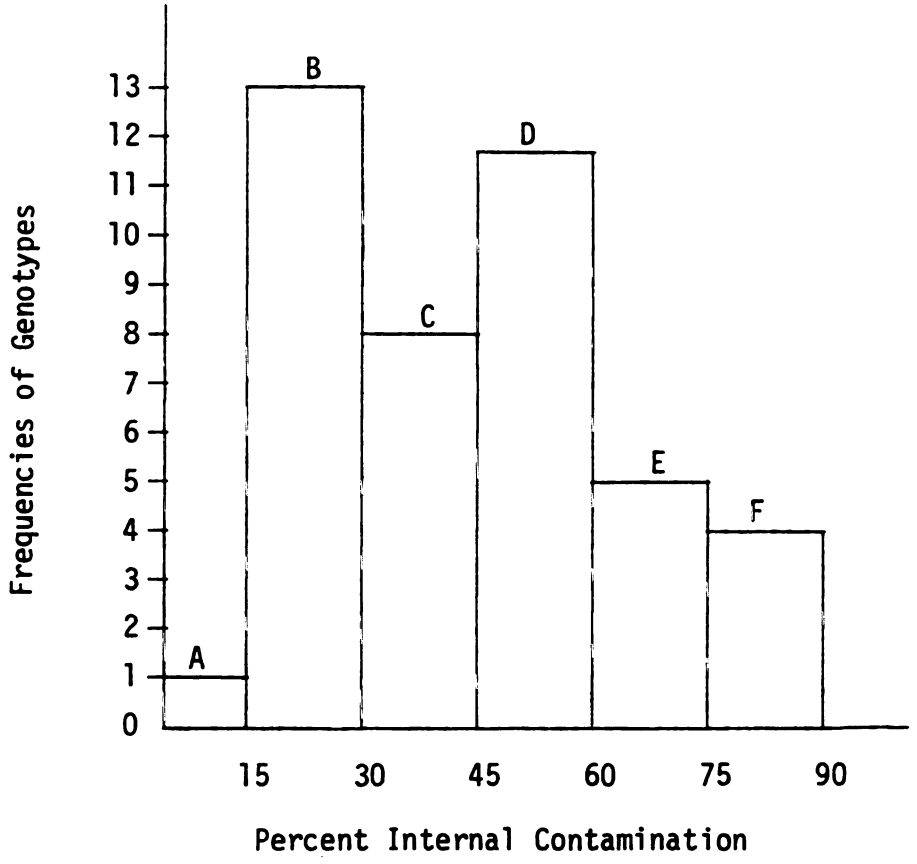


Figure 2. Genotypic variation in percent internal seed contamination by fungi in forty-two genotypes

*Most of the colored genotypes included under A, B, C are black seeded and under D, E, F are pink and brown seeded.

Genotypes	
Colored	White
A = 100%	0%
B = 80%	20%
C = 75%	25%
D = 100%	0%
E = 0%	100%

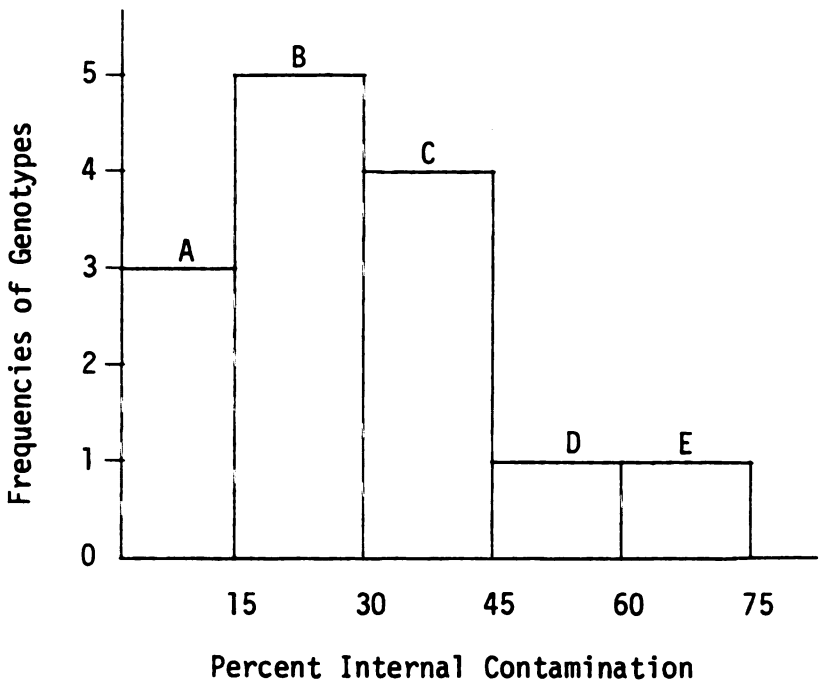


Figure 3. Genotypic variation in percent internal seed contamination by fungi in fourteen selected genotypes

Table 2. Percentage internal infection of dry bean (*Phaseolus vulgaris* L) seeds by fungi

Genotype	Seed coat color	Percent infection (20 seeds)
San-Fernando (S-182 N)	black	0
Jalpatagua 72	black	20
Collection 168N	black	20
Pecho Amarrillo	black	20
Jamapa	black	20
Linea 29	black	20
R-345 OZ	pink	20
Ex-Rico-23	white	20
Campbell Soup 109	white	20
M. gerais	black	30
San Pedro Pinula 72	black	30
Lamaniere	purple mottled	30
Redkloud	pink	30
Campbell Soup 107	white	30
25-M-($3F_5$)	black	40
ICA Huasano	balck	40
I-968	black	40
PI 313 868	black	40
PI 169 299	red	40
C 63 S-630-B	beige	40
Campbell Soup 105	white	40
Atlas R-9	white	40

Table 2. (Continued)

Genotype	Seed coat color	Percent infection (20 seeds)
Porrillo Sintetico	black (brownish)	50
PI 310 740	black	50
PI 201 333	black	50
R.K. 7690	pink	50
Nep-2	white	50
Mexico-12-1	dark brown	50
Brasil 2	brown	50
Campbell Soup 101	white	50
Campbell Soup 102	white	50
Campbell Soup 106	white	50
Campbell Soup 103	white	60
21-M-($3F_5$)	black	70
Tuscola	white	70
Campbell Soup 104	white	70
Campbell Soup 110	white	70
PI-196 936	brown	70
MSU Line 20489	white	80
PI 284 703	light brown	80
Nahuizaico	red	80
Sanilac	white	90

Table 3. Percentage internal infection of dry bean (*Phaseolus vulgaris* L) seeds by fungi

Genotype	Seed coat color	Percent infection (20 seeds)
San-Fernando	black	1
Brasil 2	brown	15
Jamapa	black	15
Collecion 168 N	black	20
Ex-Rico-23	white	20
Jalpatagua 72	black	30
Pecho Amarillo	black	30
Linea 29	black	30
R 345-LRK OZ	pink	35
C 63 S-630-B	beige	45
PI 169 299	red	45
Campbell Soup 109	white	45
Mexico-12-1	dark brown	50
Sanilac	white	75

CHAPTER II

SECTION A

EFFECT OF DELAYED HARVEST ON INCIDENCE OF INTERNAL SEED BORNE FUNGI IN DRY BEANS (*Phaseolus vulgaris* L)

Materials and Methods

Field Technique

Seven genotypes were grown at the Saginaw, Michigan Bean and Beet Research farm during the summer of 1978. The genotypes were as foloows:

	Genotype	color
1.	San-Fernando	black
2.	Ex-Rico-23	white
3.	Tuscola	white
4.	Nep-2	white
5.	Seafarer	white
6.	Turrialba #1	black
7.	Black Turtle Soup	black

Genotypes were used as main plots with three harvest dates as subplots within each genotypes. The harvest dates were: (a) normal crop maturity, (b) two weeks after maturity and, (c) three weeks after maturity. Thus the experiment was set up in a split-plot design with four replications. The experimental unit consisted of four five

meter rows per plot with 50 cm between rows.

Because dry hot weather prevailed as plants approached physiological maturity, the entire planting was sprayed three times at three day intervals with a heavy suspension of fungal spores obtained by washing molded pods obtained from earlier maturing beans.

Because of earlier maturity, Seafarer was harvested ten days earlier than the other genotypes. Two meters from each of the two center Seafarer rows were hand-harvested on 8 September 1978 (normal harvest). The second Seafarer harvest and the first harvest of the remaining genotypes were made in a similar manner in 19 September.

The third Seafarer harvest and the second harvest of the other genotypes were made on 10 October. The third harvest for six genotypes was made on 17 October. All harvest samples were hand-threshed and the seed stored in a cold room at 4°C until use. Moisture content of seed was not measured.

Laboratory Assay Technique

The assay technique for detection of internal seed contamination by fungi was identical to that used in 1977 except that seeds were soaked for one rather than two minutes in the 2.6% NaOCl. One hundred seeds from each treatment were tested for internal fungal contamination.

Data Analysis

The data for Seafarer harvested earlier than the other six genotypes were analysed as a randomized block design with the three harvests as treatments and four replications. The other genotypes were analysed as split-plot design.

Fungi Isolation and Identification

Fungal hyphae growing from infected seeds were transferred into fresh SPDA plates and incubated for about one week. Fungal isolates were then identified to genus with the aide of Barnett and Hunters Guide (9).

Results

There were highly significant genotypic differences at a 17.0856 F value for incidence of internal seed-borne fungi (Table 4). Delay in harvest past normal maturity did not significantly affect internal seed infection. However, pod molding increased as harvest was delayed (figs. 4, 6, 8, 10, 12, 14, 16). Genotype x harvest time interaction was highly significant indicating that some genotypes might have had a significant increase in seed infection due to the delayed harvests.

Since Analysis of Variance (Table 4) showed no significant effect of harvest date, genotypic means at each harvest date were compared to determine if there was a significant effect within genotype (Table 5). Internal seed infection in San-Fernando was not affected by harvest dates (figs. 5A, B, and C).

First harvest means were significantly different from second and third harvest means in the Ex-Rico-23, Nep-2, and Turrialba #1 genotypes; however, differences between the second and the third harvest means were not significant (figs. 9, 11, and 13). Tuscola and Black Turtle Soup showed similar results. Second and third harvest means were not significantly different from each other but differed significantly from first harvest means. Seed infection in both genotypes increased as harvest was delayed (figs. 7 and 15A, B and C).

Table 4. Effect of genotype and delayed harvest on internal bean (*Phaseolus vulgaris* L) seed infection by fungi

Analysis of Variance				
Source	df	Sum of squares	Mean square	F
Total	71	9149.523	--	
Blocks	3	228.585	76.195	
Genotypes	5	4401.089	880.2178	17.0856**
Error(a)	15	772.771	51.5180	
Harvests	2	121.850	60.925	1.5517
Genotypes X Harvests	10	2211.818	221.1818	5.6335**
Error(b)	36	1413.41	39.2613	

Note: The data were transformed by the arcsine method. Genotypes used were:

San-Fernando

Ex-Rico-23

Tuscola

Nep-2

Turrialba #1

Black Turtle Soup

Table 5. Comparison of genotypic and harvest time means for internal bean (Phaseolus vulgaris L) seed infection by fungi

A'	San-Fernando	Ex-Rico-23	Tuscola	Nep-2	Turrialba #1	BTS	Average
B'	L S D (A' in B') = 9.919						
	L S D (B' in A') = 8.954						LSD(B')= 3.656
Normal maturity	24.38 ACa	40.23 Ba	37.9 Ba	37.12 Ba	33.11 Ba	22.92 Ca	32.61 x
Two weeks after nm	21.66 ACa	27.40 ACDb	48.82 Bb	17.69 Cb	26.75 CDab	34.82 Db	29.5 x
Three weeks after nm	17.56 Aa	31.92 BDEab	51.02 Cb	20.55 Ab	24.35 Db	36.97 Eb	30.39 x
Average	21.2V	33.18 W	45.91 X	25.12 VY	28.07 WYZ	31.57 WYZ	
	LSD(A') = 6.244						

Note: A' = Genotypes; B' = Harvest dates; BTS = Black Turtle Soup. Capital letters are used to compare the row means and the small letters for the columns means. Means followed by the same capital letters in the rows and the same small letters in the columns are not significantly different from each other at P = 0.05.

Figure 4. Pod molding in San-Fernando bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

Figure 5A. Internal fungal infection of seed of San-Fernando genotype harvested at normal maturity.

Figure 5B. Internal fungal infection of seed of San-Fernando genotype harvested three weeks after normal maturity.

Figure 5C. Internal fungal infection of seed of San-Fernando genotype harvested three weeks after normal maturity.

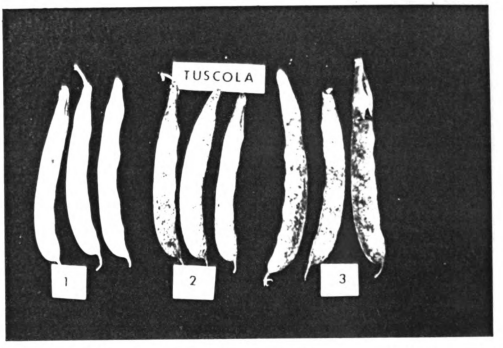


Figure 6. Pod molding in Tuscola bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

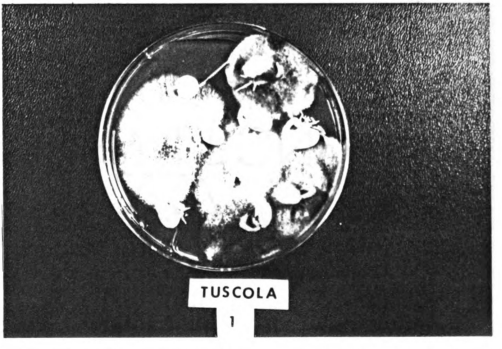


Figure 7A. Internal fungal infection of seed of Tuscola genotype harvested at normal maturity.

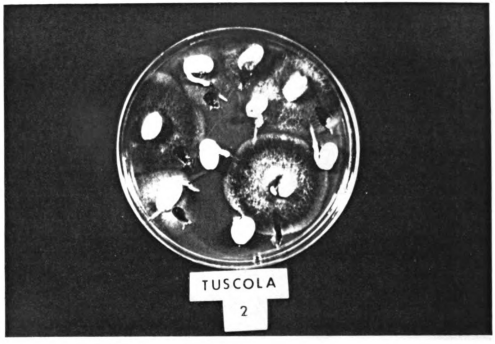


Figure 7B. Internal fungal infection of seed of Tuscola genotype harvested two weeks after normal maturity.

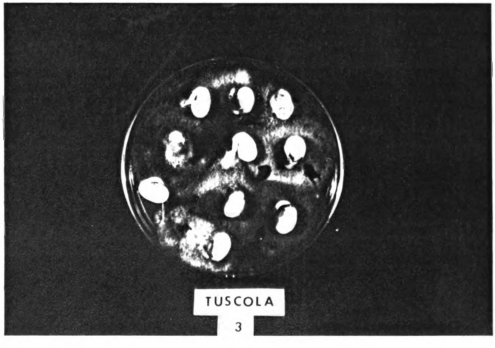


Figure 7C. Internal fungal infection of seed of Tuscola genotype harvested three weeks after normal maturity.

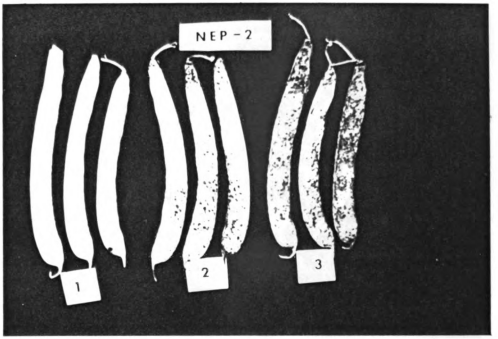


Figure 8. Pod molding in Nep-2 bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

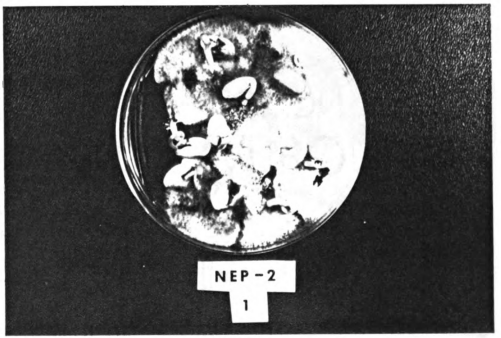


Figure 9A. Internal fungal infection of seed of Nep-2 genotype harvested at normal maturity.

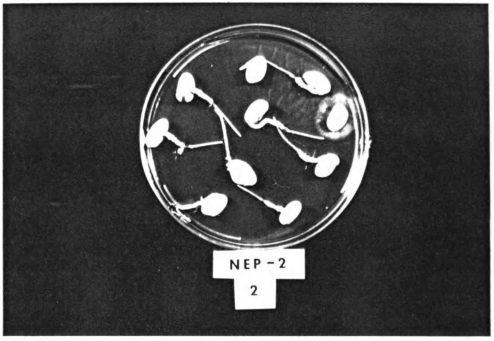


Figure 9B. Internal fungal infection of seed of Nep-2 genotype harvested two weeks after normal maturity.

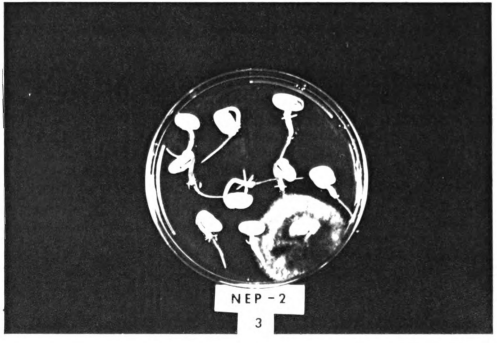


Figure 9C. Internal fungal infection of seed of Nep-2 genotype harvested three weeks after normal maturity.

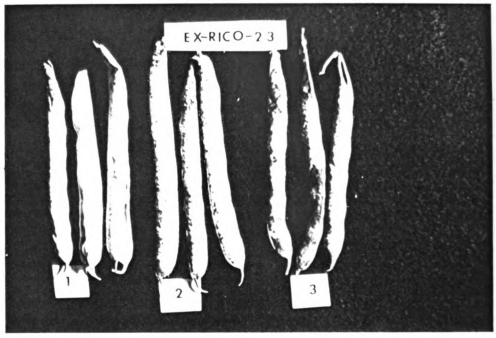


Figure 10. Pod molding in Ex-Rico-23 bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

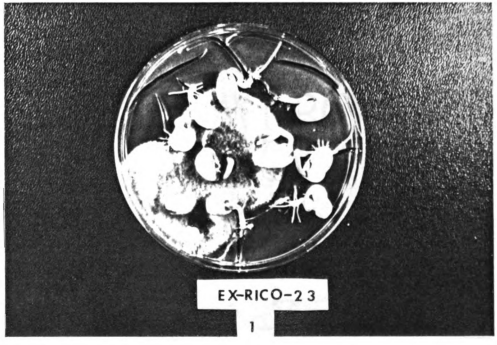


Figure 11A. Internal fungal infection of seed of Ex-Rico-23 genotype harvested at normal maturity.

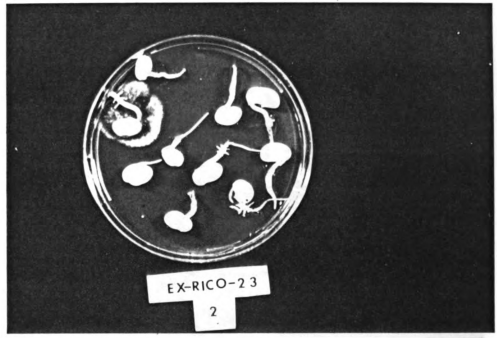


Figure 11B. Internal fungal infection of seed of Ex-Rico-23 genotype harvested two weeks after normal maturity.

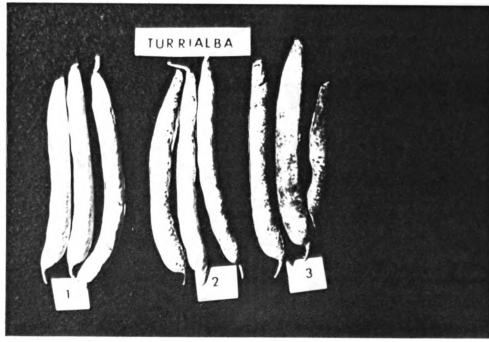


Figure 12. Pod molding in Turrialba #1 bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

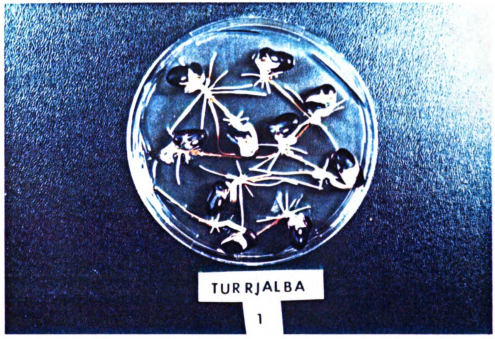


Figure 13A. Internal fungal infection of seed of Turrialba #1 genotype harvested at normal maturity.

Figure 13B. Internal fungal infection of seed of Turrialba #1 genotype harvested two weeks after normal maturity.

Figure 14. Pod molding in Black Turtle Soup (BTS) bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

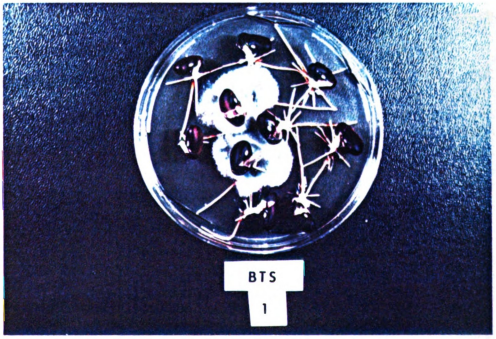


Figure 15A. Internal fungal infection of seed of Black Turtle Soup genotype harvested at normal maturity.

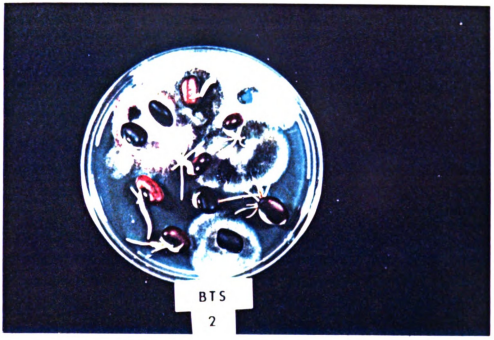


Figure 15B. Internal fungal infection of seed of Black Turtle Soup bean genotype harvested two weeks after normal maturity.

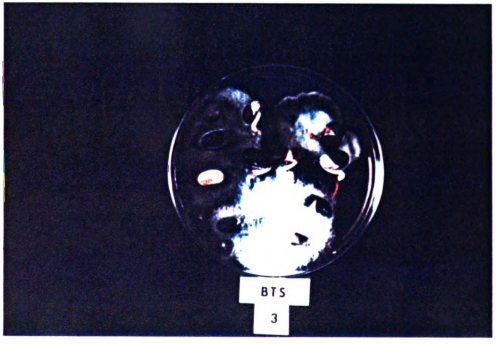


Figure 15C. Internal fungal infection of seed of Black Turtle Soup genotype harvested three weeks after normal maturity.

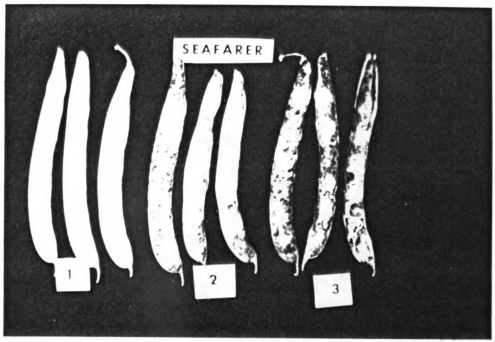


Figure 16. Pod molding in Seafarer bean genotype harvested at normal maturity (1), two weeks after (2), and three weeks after (3) normal maturity.

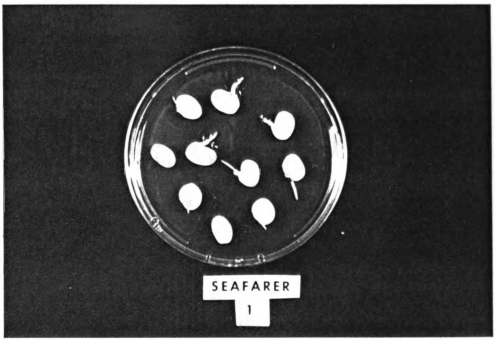


Figure 17A. Internal fungal infection of seed of Seafarer genotype harvested at normal maturity.

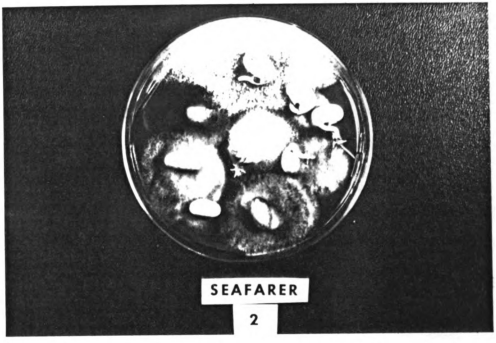


Figure 17B. Internal fungal infection of seed of Seafarer genotype harvested two weeks after normal maturity.

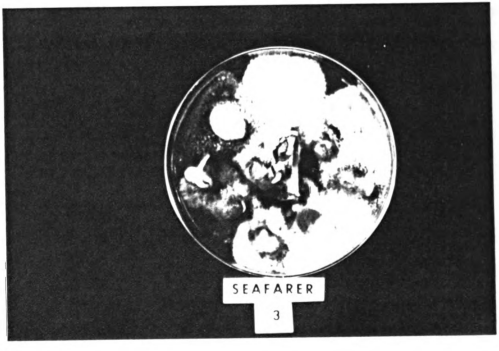


Figure 17C. Internal fungal infection of seed of Seafarer genotype harvested three weeks after normal maturity.

Comparison of the six genotypic means at normal maturity showed significant variation (Table 5). San-Fernando and Black Turtle Soup were not significantly different from each other, but differed significantly from Ex-Rico-23, Tuscola, Nep-2, and Turrialba #1.

Comparison of the genotypic means at second harvest showed several significant differences. San-Fernando, Ex-Rico-23, Nep-2, and Turrialba #1 were not found significantly different from each other. Infection in Tuscola was significantly higher than in other five genotypes. Ex-Rico-23, Turrialba #1, and Black Turtle Soup were not significantly different; however, theinfection in Black Turtle Soup was significantly higher than in San-Fernando and Nep-2.

Comparison of genotypic means at third harvest showed that San-Fernando and Nep-2 were not significantly different from each other. They differed significantly from Ex-Rico-23, Tuscola, Turrialba #1, and Black Turtle Soup. Turrialba #1 differed significantly from Black Turtle Soup and Tusoola. Ex-Rico-23 differed significantly from Tuscola. Black Turtle Soup and Ex-Rico-23 did not differ significantly from each other. Infection in Tuscola was the highest and differed significantly from Black Turtle Soup which showed the second highest level of infection.

Average means comparison of six genotypes at three different harvest dates did not show any significant effect of harvest date. Similarly the average means comparison of the three harvest times in six genotypes showed genotypic variation in rate of seed infection. San-Fernando and Nep-2 did not differ significantly from each other and neither did Ex-Rico-23, Turrialba #1 and Black Turtle Soup. The

average infection in Tuscola was the highest among six genotypes and was significantly different.

Seafarer, a white seeded genotype showed a highly significant effect of delay in harvest (Table 6); the F value was 1769.500. The three harvest means were compared and showed a linear increase in seed infection from 0.25% to 77.75% to 88.25%, at first, second and third harvest dates, respectively (Table 7).

Table 6. Effect of delayed harvest on internal fungal infection of seed of 'Seafarer' bean

Analysis of Variance				
Source	df	Sum of squares	Mean square	F
Total	11	18550.917	--	
Blocks	3	38.917	12.972	
Harvest dates	2	18480.667	9240.333	1769.500**
Error	6	31.333	5.222	

Table 7. Comparison of three harvest time means for internal fungal infection of seed of 'Seafarer'

Harvest dates	Means
Normal maturity	0.25 a
Two weeks after normal maturity	77.75 b
Three weeks after normal maturity	88.25 c
LSD 0.05	3.952

Means followed by the same letter are not significantly different from each other at P = 0.05 by Duncans multiple range test.

Incidence of total fungi isolated from surface-sterilized dry bean seed and occurrence of fungi by genera are presented in Table 8. Fungi isolated most frequently were: Alternaria sp and Rhizoctonia sp. Fungi isolated less frequently included Fusarium sp, Penicillium sp, Epicoccum sp, Cladosporium sp, Chaetomium sp, Rhizopus sp, and several other unidentified isolates.

Alternaria sp was isolated most frequently from Tuscola seed, followed by Turrialba #1, relatively few Alternaria sp were isolated from San-Fernando, Nep-2, Ex-Rico-23, and Black Turtle Soup. Incidence of Rhizoctonia sp was highest in San-Fernando, and lowest in Tuscola and Turrialba #1. Incidences of internal seed infection by Rhizoctonia sp in Ex-Rico-23, Nep-2, and Black Turtle Soup were almost equal. Alternaria sp, and Rhizoctonia sp showed some genotypic specificity while Fusarium sp and other fungi did not.

San-Fernando: Occurrence of Alternaria sp increased from 61.4 to 63.6 to 75.0% as harvest was delayed from normal maturity to two weeks and to three weeks after normal maturity, respectively. However, incidence of total fungi was decreased by delayed harvest. Occurrence of Rhizoctonia sp in this genotype decreased with delay in harvest.

Ex-Rico-23: Incidence of Alternaria sp increased from 64.5 to 71.9 to 72.8% at normal maturity, two weeks after and three weeks after normal maturity, respectively. On the other hand, total incidence and occurrence of Rhizoctonia decreased from 32.5 to 25.8, to 17.5% at the same harvest times.

Tuscola: Incidences of Alternaria sp and Rhizoctonia sp were very similar at all harvest dates. The highest occurrence of Alterneria and Rhizoctonia in this genotype were 89.4 and 14.2%

Table 8. Incidence of total internal fungi and fungal genera in seeds of six dry bean (Phaseolus vulgaris L) genotypes[1]

Genotypes	Harvest dates[3]	Total fungi	Incidence of fungi by genera in percent[2]								
			Alt.	Rhiz.	Fus.	Pen.	Epi.	Clad.	Chaet.	Rhp.	Misc.
San-Fernando	1	17.5	61.4	37.7	1.4	0	0	0	0	0	1.4
	2	13.8	63.4	30.9	1.8	0	2.8	0	1.8	0	0
	3	9.0	72.0	22.2	0	2.8	0	0	0	0	2.8
Ex-Rico-23	1	42.3	64.5	32.5	0	1.8	0	0	0	1.8	0
	2	22.3	71.9	25.8	0	0	0	0	0	0	2.2
	3	28.5	72.8	17.5	4.4	0	2.6	0.9	0	0	0
Tuscola	1	38.3	85.6	13.7	0	0	0	0	0	1.3	0
	2	56.5	89.4	7.6	2.7	0	0	0	0	0	0
	3	59.8	83.7	14.2	0.8	0	1.7	0.4	0	0	0
Nep-2	1	36.5	67.1	30.1	0	0.7	0	0.7	0	1.4	0
	2	9.3	78.4	21.6	0	0	0	0	2.7	0	0
	3	12.5	72.0	24.0	2.0	0	0	2.0	0	0	0
Turrialba #1	1	31.3	89.6	5.6	0	1.6	0	0	0	0	0
	2	20.5	81.7	12.2	2.4	0	2.4	0	1.2	0	0
	3	17.0	77.9	20.6	0	0	1.5	0	0	0	1.5
Black Turtle Soup	1	15.3	77.5	19.7	0	1.6	0	0	0	1.6	0
	2	33.3	75.2	21.1	5.3	0	0	0	0	0	0
	3	36.3	57.2	38.6	1.4	0.7	0.7	0.7	0	0	0.7

[1]Data obtained from 400 seeds/genotype/harvest date.

[2]Alt = Alternaria; Rhiz = Rhizoctonia; Fus = Fusarium; Pen = Penicillium; Epi = Epicoccum; Clad = Cladosporium; Chaet = Chaetomium; Rhp = Rhizopus; Misc = Miscellaneous unidentified fungi.

[3]1 = Seeds harvested at normal crop maturity; 2 = seeds harvested two weeks after 1; 3 = seeds harvested three weeks after 1.

respectively.

Nep-2: Incidence of Alternaria sp increased from 67.1 to 78.4% as harvest was delayed to two weeks after normal maturity and then decreased to 72.0% at the third harvest date. Rhizoctonia sp did not show any change in incidence due to harvest time. Cladosporium spp were isolated most frequently in this genotype.

Turrialba #1: This genotype exhibited a specific harvest date response to incidence of Alternaria sp and Rhizoctonia sp. Alternaria sp decreased from 89.6 to 81.7 to 77.9% as harvest was delayed from normal maturity to two and three weeks after normal maturity, respectively. However, Rhizoctonia sp increased from 5.6 to 12.2 to 20.6% for the same harvest dates.

Black Turtle Soup: This genotype was similar to Turrialba #1. Occurrence of Alternaria sp decreased from 77.5 to 75.2 to 57.24% as harvest was delayed. Rhizoctonia sp increased from 19.7 to 21.1 to 38.6% due to delay in harvest.

SECTION B

PRELIMINARY OBSERVATIONS ON THE NATURE OF INHERITANCE FOR RESISTANCE TO INTERNAL SEED-BORNE FUNGI IN DRY BEAN (*Phaseolus vulgaris* L)

Materials and Methods

Seeds of bean cultivars San-Fernando (black seeded) and Tuscola (white seeded) harvested during the Fall of 1977 were taken from bulk seed sources and planted in a green-house at Michigan State University for making hybrids. San-Fernando and Tuscola were considered as resistant and susceptible parents respecitvely on the basis of results obtained from the internal fungal seed infection test conducted in 1977. Reciprocal crosses were made in the green-house during the winter of 1978.

Seeds containing F_1 germs and of the parents were planted at the Saginaw Michigan Bean and Beet Research Farm during the summer of 1978. Only the parents were replicated, with four rows in each plot. They were sprayed at physiological maturity with heavy suspensions of mold spores, as mentioned in Chapter II, Section A. All F_1s and two rows of the parents were hand harvested two weeks after normal maturity. Seeds were stored at about 4°C until tested.

Two hundred seeds from each of fifteen F_1 plants were taken for the internal fungal seed contamination test. Four hundred seeds from

each parent were used for the fungal infection test. The laboratory technique was the same as mentioned in Chapter I and Chapter II, Section A.

Degree of internal seed infection by fungi was expressed in percentages for the genetic study.

Results

Internal seed infection of fifteen F_1 plants showed great plant to plant variation (fig. 18), indicating major environmental effects. Infection in seed of the fifteen plants ranged from 7.5 to 57.5%, which is less than the mean percentage infection in the resistant parent but more than the mean percentage infection in the susceptible parent respectively (Table 9).

Variation in degree of internal seed infection between the F_1 plants is thought due to two main factors:

(1) Stage of plant development and maturation was not identical for all F_1 plants. But all were harvested at the same time irrespective of stage of maturity. Plants which germinated, developed, and matured earlier were exposed to hot wet weather for a relatively longer period after normal maturity than those which did not germinate and mature early. Differences in the stage of maturity and duration of exposure to hot wet weather caused great variation. This conclusion is supported by the trend of internal seed infection in Tuscola and San-Fernando. Delayed harvest after normal maturity decreased seed infection in San-Fernando but infection was increased in Tuscola.

(2) Difference in plant architecture may have caused variation in extent of seed infection within the genotype. Percent internal

Figure 18. F_2 germs of the cross between San-Fernando and Tuscola showing highest (A) and lowest (B) internal fungal infection of seed of two F_1 plants.

Table 9. Percentage internal fungal seed infection of fifteen F_1 plants of the cross between 'San-Fernando' and 'Tuscola' bean genotypes

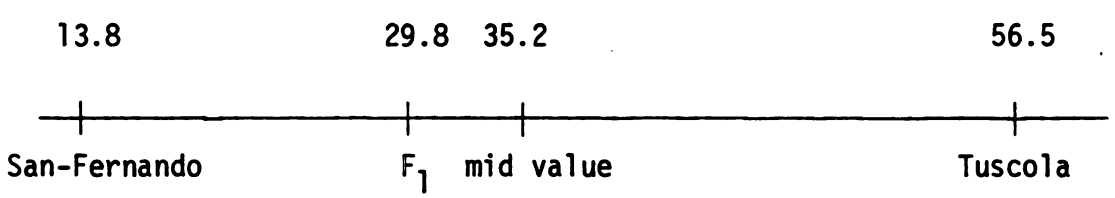
Percent infection (200 seeds in F_1		Percent infection in parents (400 seeds in each)	
	57.5		
	54.0		
	47.5		
	36.36		
	31.5		
	31.19		
	29.0		
	27.5		
	27.5		
	26.94		
	21.13		
	21.0		
	14.5		
	13.5	San-Fernando	Tuscola
	7.5		
Mean	29.77	13.75	56.5

seed infection by fungi among dry bean cultivars with pods in contact with soil (41) is always higher than among cultivars with pods not in contact with soil. There are distinct variation in plant vigor among the fifteen F_1 plants; some were more vigorous and lodged with many pods in contact with soil, resulting in high percentages of mold invasion in seed. Other plants were less vigorous and erect with no pods in contact with soil, resulting in less infection.

Further speculation on the fact the fifteen F_1s showed such a range of infection can be illustrated as follows:

Assume San-Fernando carries dominant genes for resistance to (say) Penicillium and recessive genes for resistance to (say) Rhizopus, then we would expect the F_1 to be resistant to Penicillium and susceptible to Rhizopus. Now, suppose that in the field the natural distribution of Penicillium and Rhizopus was non-random or irregular so that some F_1 plants were exposed only to Penicillium spores, in which case those particular plants would show resistance, and some F_1 plants would be exposed only to Rhizopus spores, in which case those F_1s would show susceptibility like Tuscola.

The mean percentage infection of fifteen F_1, San-Fernando and Tuscola were 29.8, 13.8, and 56.5% respectively:



Very provisionally, it may be suggested that genes affecting seed infection under these conditions display additivity to slightly partial dominance for the resistant response. This is indicated by the fact that mean percentage seed infection in F_1 was only slightly

displaced from the mid parental value and towards the resistant parent. Resistance cannot be said to be specific for there are many fungi which are internally seed-borne. Since the F_1 plants had black seed coats and yet showed infection as high as 57%, resistance may be independent of seed coat color.

CHAPTER III

SECTION A

EFFECT OF DELAYED HARVEST ON LABORATORY SEED GERMINATION IN DRY BEAN (*Phaseolus vulgaris* L) GENOTYPES

Materials and Methods

Seed samples of the seven genotypes which had been harvested at three different times in the fall of 1978 were stored at about 4°C until April 1979. Germination tests were performed in a germinator maintained at 26 ± 1°C. Kimpack, a germination media, was placed over wax paper in a germination tray.

Four replications of 100 seeds each for each genotype and each harvest date were tested. Media was lightly watered and 200 seeds were placed on each tray. Seeds were covered with wet paper towelling. Seed germination was counted after 7 days of incubation.

Criteria of Germination Counts

Seedlings which possessed a) strong primary roots and/or secondary roots (fig. 19) sufficiently healthy to support the seedling and b) long hypocotyls with at least half of both cotyledons and with normal plumule were considered to have germinated in a normal manner. Abnormal germination types included a) decayed seeds, and b) seedlings with very short and thickened hypocotyls with no strong primary or

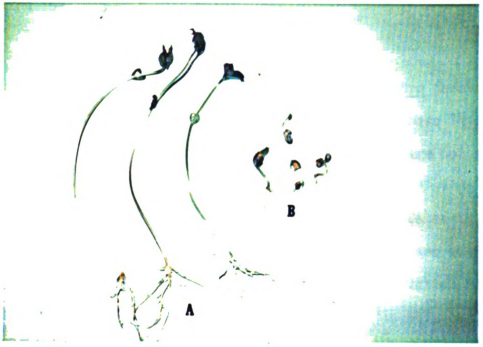


Figure 19. Normal (A) and abnormal (B) dry bean seedlings in germination test. Abnormal seedlings developed from seed internally infected with fungi.

secondary roots, and no plumule.

Data Analysis

Since results were taken as percent germination, data were transformed by the arcsin method. Six genotypes were analysed in split-plot, and Seafarer as a randomized block.

Results

Genotypes showed significant variation for *in vitro* germination. Delay in harvest did not result in any significant decrease in *in vitro* germination (Table 8). Genotype-harvest interactions were not significant.

Comparison of germination means at three harvest dates in six genotypes is presented in Table 9. All six genotypic means at first and second harvest did not differ significantly. Germination in Tuscola seed from the third harvest decreased significantly compared to the other five genotypes.

Average germination means of six genotypes over three different harvest dates did not show any significant reductions in seed germination. However, average germination means of the three harvest dates over six genotypes showed genotypic variation. Delay in harvest past normal maturity caused significant reduction in laboratory seed germination only in Tuscola.

Seed germination in Seafarer for the three harvest dates did not show any significant differences due to delay in harvest (Table 10). However, harvest means comparisons (Table 11) showed significant reduction in germination from first harvest to the third harvest suggesting that delay in harvest may cause germination reduction in this

genotype.

Table 10. Effect of genotype and delayed harvest on laboratory seed germination of dry bean (Phaseolus vulgaris L) seeds

Analysis of Variance				
Source	df	Sum of squares	Mean square	F
Total	71	3202.6664	--	
Blocks	3	156.4657	52.155	
Genotypes	5	570.7594	114.152	7.161**
Error (a)	15	239.1269	15.942	
Harvests	2	119.6421	59.820	1.162NS
Genotypes X Harvests	10	264.9426	26.494	0.51NS
Error (b)	36	1851.7297	51.437	

Data are transformed by the arcsin method.

Table 11. Comparison of genotypic and harvest time means for laboratory seed germination of dry bean (Phaseolus vulgaris L) seeds

A'	San-Fernando	Ex-Rico-23	Tuscola	Nep-2	Turrialba #1	BTS	
B'	LSD (A' in B') = 9.483 LSD (B' in A') = 10.249						LSD = 4.184
First	90.0 Aa	87.13 Aa	83.64 Aa	87.13 Aa	88.57 Aa	90.0 Aa	87.74 x
	88.57 Aa	80.67 Aa	82.24 Aa	87.51 Aa	82.98 Aa	82.02 Aa	84.49 x
	90.0 Aa	86.53 Aa	75.47 Ba	86.77 Aa	87.13 Aa	87.13 Aa	85.51 x
	89.52 V LSD = 3.474	84.78 W	80.45 X	87.14 VW	86.23 VW	87.38 VW	

Note: Data are transformed by the arcsine method. A' = Genotypes; B' = Harvest dates; BTS = Black Turtle Soup. Capital letters are used to compare the row means and small letters for the comparison of the column means. Means followed by the same capital letters in the rows and the same small letters in the columns are not significantly different at P = 0.05.

Table 12. Effect of delayed harvest on laboratory seed germination of Seafarer bean seed

Analysis of Variance				
Source	df	SS	MS	F
Total	11	658.144	--	
Block	3	131.973	43.99	
Harvests	2	264.276	132.138	3.027 NS
Error	6	261.895	43.649	

Table 13. Comparison of harvest time means in Seafarer bean genotype for laboratory seed germination

	Means
First harvest	90 a
Second harvest	84.30 ab
Third harvest	78.50 b
LSD (0.05)	11.4315

Means with the same letter in common are not significantly different from each other at P = 0.05.

SECTION B

EFFECT OF DELAYED HARVEST ON FIELD EMERGENCE IN DRY BEAN (*Phaseolus vulgaris* L) SEED

Materials and Methods

Seven genotypes as indicated in Chapter II, Section A were harvested at three different dates in the fall of 1978 and were used for field emergence tests. Seeds were planted at the Crop Science Research Farm, East Lansing, Michigan on 14 June 1979. Genotypes were used as main plots with three different harvest dates on sub-plots within each genotype. Thus the experiment was a split plot design with four replications. The experimental unit consisted of two four meter rows with 50 seeds/row.

Because hot dry weather prevailed before and after planting, rows were manually watered at five days of planting. No additional watering was necessary thereafter. Number of seeds emerged were counted 20 days after planting.

Seafarer was analysed as a random block and remaining genotypes (San-Fernando, Ex-Rico-23, Tuscola, Nep-2, Turrialba #1, and Black Turtle Soup) as a split-plot design. Data were obtained as percentage emergence and transformed by the arcsin method.

Results

There was highly significant genotypic variation for field emergence (Table 14). Delay in harvest did not have any significant effect; genotype x harvest date interactions were also not significant.

Table 14. Effect of genotype and delayed harvest on field emergence of dry bean (*Phaseolus vulgaris* L) seeds[1]

Analysis of Variance				
Source	df	Sum of squares	Mean square	F
Total	71	3402.602	--	
Blocks	3	279.280	93.09	
Genotypes	5	1229.786	245.95	5.58**
Error(a)	15	661.217	44.08	
Harvest dates	2	31.086	15.54	0.54 NS
Genotypes X Harvest	10	179.588	17.95	0.63 NS
Error (b)	36	1021.042	28.36	

[1]Data are transformed by the arcsin method.

Comparison of genotypic and harvest time means is presented in Table 15. San-Fernando and Turrialba #1 did not differ significantly from each other but showed significantly higher field emergence than Nep-2 for seed harvested at normal maturity. Ex-Rico-23, Tuscola, Nep-2, and Black Turtle Soup did not differ significantly from each other for field emergence for seed harvested at normal maturity.

San-Fernando, Turrialba #1, and Black Turtle Soup were not significantly different from each other but had significantly higher

Table 15. Comparison of genotypic and harvest time means for field emergence of dry bean (*Phaseolus vulgaris* L) seeds[1]

A'	San-Fernando	Ex-rico-23	Tuscola	Nep-2	Turrialba #1	BTS	Average
B'	LSD (A' in B') = 8.715 LSD (B' in A') = 7.610						LSD = 3.107
Normal maturity	65.39 Aa	62.24 ABa	60.65 ABa	55.52 Ba	65.01 Aa	62.59 ABa	
Two weeks after normal maturity	65.12 Aa	59.20 ABa	57.56 ABa	56.27 Ba	67.53 Aa	67.60 Aa	62.21 x
Three weeks after normal maturity	66.07 Aa	55.12 Ba	59.86 ABa	53.30 BCa	66.12 Aa	63.57 Aa	60.67 x
Average	65.52 X	58.85 Y	59.35 Y	55.03 Y	66.22 X	64.58 X	
	LSD (A') = 5.776						

[1]Data are transformed by the arcsin method. A' = Genotypes; B' = Harvest dates; BTS = Black Turtle Soup. Capital letters are used to compare the row means and small letters for the comparison of the column means. Means followed by the same capital letters in the rows and the small letters in the columns are not significantly different from each other at P = 0.05.

field emergence than Nep-2 for seed harvested two weeks after normal maturity. Ex-Rico-23, Tuscola, and Nep-2 again did not differ significantly from each other.

Comparison of genotypic means for field emergence for seed harvested three weeks after normal maturity showed no significant differences among Tuscola, Ex-Rico-23, and Nep-2. San-Fernando, Turrialba #1, and Black Turtle Soup had higher emergence than Nep-2, Tuscola and Ex-Rico-23.

Average mean comparison of six genotypes over three different harvest dates showed general superiority of black seeded types for field emergence. All three black seeded genotypes (San-Fernando, Turrialba #1, and Black Turtle Soup) had significantly higher field emergence than white seeded types.

Mean comparison of three different harvest dates within individual genotypes did not show any significant effect of delayed harvest on field emergence. Similarly, average mean comparison of three different harvest dates over six genotypes did not show significant effect of harvest dates for field emergence.

Seafarer, a white seeded genotype showed a significant effect of delayed harvest on field emergence at the 5 percent level of significance (Table 16). Comparison of the three different harvest date means (Table 17) showed no significant difference between the seeds harvested at normal maturity and two weeks after normal maturity for field emergence. However, field emergence decreased significantly at the third harvest suggesting that delay in harvest decreased field emergence in this genotype.

Table 16. Effect of delayed harvest on field emergency of Seafarer seed[1]

Analysis of variance			
Source	df	Sum of squares	Mean square F
Total	11	374.161	--
Blocks	3	119.744	39.91
Harvest dates	2	168.330	84.165 5.873*
Error	6	14.33	

[1]Data are transformed by the arcsin method.

Table 17. Comparison of harvest time means in Seafarer genotype for field emergence

Harvests	Means
Normal maturity	61.08 a
Two weeks after normal maturity	63.29 a
Three weeks after normal maturity	54.47 b
LSD .05	6.550

Means followed by the same letter are not significantly different from each other.

DISCUSSION

Pod and seed molding in dry bean (Phaseolus vulgaris L) are not equal in all genotypes, and there is genotypic variation for the extent of molding. Molding is favored by hot humid or wet weather after normal plant maturity. Degree of pod molding in all genotypes increased as the plants were exposed to hot wet weather after maturity (figs. 4, 6, 8, 10, 12, 14, and 16). Heavily molded plants and pods are characterized by a brownish-black covering composed of mold growth and spores.

There was no genotypic variation for the extent of external seed contamination by fungi and this is best explained by the fact that external seed contamination by fungi takes place during the process of harvesting, threshing, and seed handling (120). Healthy appearing seed may carry 100% external contamination. These results suggest no possibility of obtaining seed free from externally seed-borne fungi when weather favors mold growth. Seeds already contaminated with fungal spores start developing mold when temperature and humidity are high in storage. High correlations between moisture content and duration of storage have been reported (13,18,120).

There is considerable genotypic variation for percent incidence of internal seed infection by fungi. Results obtained from a preliminary screening test in 1977 indicated that San-Fernando and Sanilac were the most resistant and most susceptible genotypes, respectively,

for internal seed infection by fungi. Genotypes with pigmented seed coat colors, particularly black were generally more resistant than white seeded genotypes (Tables 2 and 3).

Varietal differences for the degree of mold invasion have been reported in bean (Phaseolus vulgaris L) (18), soybean (Glycine max) (38,50,104,138,154,162) and pigeon pea (Cajanus cajan) (140). We also found significant differences for internal seed infection by fungi among seven dry bean genotypes. Consistent genotypic variation suggested that genetic factors controlled resistance.

Delay in harvest after normal maturity did not affect all genotypes in the same manner. Many researchers (1,40,104,113,133,157) have reported that incidence of internal seed-borne fungi increases as harvest is delayed. Our results differed from these reports; different dry bean genotypes responded differently. Internal infection in Seafarer, Black Turtle Soup and Tuscola increased as harvest was delayed beyond normal maturity. However, infection in San-Fernando (black), Nep-2 (white seeded mutant from San-Fernando), and Turrialba #1 (black seeded) decreased as harvest was delayed. These results suggest that seed of all genotypes of dry bean harvested at normal maturity under wet weather are not necessarily high quality in relation to internal fungal seed infection.

High temperature and humidity during the period of crop maturity are believed responsible for increased molding. Plants matured under dry weather conditions and harvested at normal maturity produce seed of excellent quality. Results with seed of susceptible Seafarer, harvested at normal maturity showed essentially no internal fungal infection. However, Seafarer seed harvested two and three weeks after

normal maturity was heavily infected internally with fungi.

There are specific genotype x fungus interactions. Alternaria sp followed by Rhizoctonia spp dominate other fungi and are the most commonly isolated fungi in dry beans grown at Saginaw Michigan. The two fungi averaged gerater than 70 percent seed incidence. Tuscola and San-Fernando were the most and least congenial hosts for occurrence of Alternaria species, respectively. San-Fernando and Tuscola were the most and least preferred hosts for the occurrence of Rhizoctonia species, respectively.

Species of Cladosporium, Fusarium, Epicoccum, Chaetomium, Rhizopus, and Penicillium, were also internally seed-borne in dry bean grown in Michigan. This is the first report of Epicoccum, and Chaetomium spp as internal contaminants in dry bean seed.

It is believed that different bean genotypes may synthesize different kinds of organic compounds which either inhibit or stimulate the development of a particular fungal species.

Resistance of bean plants to fungal seed and seedling pathogens has been reported to be associated with colored seed coat (29,30,87, 109,110,111,141). Pigmented scales in onion confer resistance to smudge (Colletotrichum lindemuthianum) (82). Sorghum cultivars with pigmented testa are also reported to have favorable traits such as bird resistance, inhibition of preharvest seed germination, and weathering resistance. Such pigmentation is due to the presence of phenolic compounds. The superiority of pigmented genotypes for several favorable traits is related to phenolic compounds such as tannic and/or shikimic acids (4,11,49,66,87,109,141). Our results show that some white seeded genotypes such as Nep-2, and Ex-Rico-23 are as resistant

as the black seeded genotype from which they were developed through mutation. Several black seeded genotypes, particularly Black Turtle Soup, are as susceptible to fungal infection as white seeded lines. Therefore pigmentation is not the sole determinant of resistance in dry beans to internal seed-borne fungi.

That some compound(s) may be involved in resistance to internal seed-borne fungi is suggested by the decreasing and increasing trends of infection due to delays in harvest. However, this hypothesis remains to be tested.

Phenol, a compound which could condition resistance to internal seed-borne fungi is known to be under genetic control (21,52,66,71,86, 116) and its presence in seed is heritable (31,94,95). Deakin et al. (29) reported that attempts to obtain white seeded snap bean lines with resistance to Rhizoctonia solani were not successful due to epistatic effects. The authors hypothesized that one homozygous (pp) recessive gene blocks the pathway of synthesis of phenolic ocmpounds such as phaseolin and shikimic acid. However, phaseolin is colorless and one should be able to develop white seeded lines with a level of phaseolin high enough to confer good disease resistance and seedling vigor (28). Yu Ma et al. (161) found that white seeded strains of dry beans contained no detectable amounts of tannin and that, when present in colored seeds, tannin was located in the testae. Although dark colored seeds of Phaseolus vulgaris L contained the highest levels of tannin, the authors found no strong relationship between tannin content and seed coat color. Heritability studies indicated a high broad sense heritability for tannin content.

Phenolic compounds, colored or colorless, are generally found

in the form of tannic acids, schikimic acids and phaseolin. Genotypes resistant to internal seed infection by fungi perhaps contain high level of phenolic compounds. Since the compound is in seed coat and fungi get in the seed, the mechanism of phenolics for fungal growth inhibition in seed is not well understood. However, it is speculated that fungal mycelia growing in seed produce certain metabolities which hydrolyse the phenolic compounds present in seed coat and the hydrolysed product diffuses from seed coat into the cotyledon and becomes inhibiting.

Smaller seeded genotypes have been reported to contain fewer internally seed-borne fungi than large seeded ones (104). This is explained by the fact that most tannin is located in the seed coat and smaller seed usually have more seed coat area, by weight, than large seed, and therefore may also have a higher tannin concentration. However, correlations between tannin content and seed size were not significant (161) suggesting that tannin content is independent of seed size. In the present study, San-Fernando and Nep-2 were relatively small seeded compared to other genotypes and showed less internal infection by fungi. Nevertheless, large seeded Turrialba #1 showed less infection than the smaller seeded Tuscola and Seafarer.

Results from the preliminary genetic study suggests the possibility of breeding for resistance to internal seed infection by fungi. If resistance is caused by one or more phenolics in the seed-coat or cotyledons and resistance is concentration dependent, then the F_1 which shows approximately mid-parental response does so because it is producing phenolics at about half the rate or level of the resistant parent.

It would also be necessary to assume that, since San-Fernando shows resistance to several fungi at the same time, its level of phenolics and the particular array or classes of phenolics produced confer generalized resistance. Many pairs of genes for resistance are postulated, so it should be possible to develop white seeded dry bean lines with sufficient genes to confer acceptable resistance. This conclusion is supported by the results obtained with the two isogenic lines San-Fernando (black) and Nep-2 (white) which showed equal levels of internal seed infection.

Bean genotypes with hard, thick, and intact seed coats or with thin soft seed coat were reported to exhibit resistance or susceptibility to fungal infection respectively (78). This is not the case with Nep-2 (thin soft seed coats) and Black Turtle Soup (thick, intact, seed coats). Nep-2 had less internal infection than Black Turtle Soup. Resistance therefore is not totally determined by the seed coat but by the seed itself. Internal fungal infection in seeds harvested from F_1 plants further supports this conclusion; all of the seeds (F_2 germs) possessed thick seed coats, were black, and showed internal infections as high as 57 percent.

If a genetically controlled, colorless, phenolic compound such as tannin does control resistance, the commercial value of dry bean types containing this compound might be reduced. This is because tannins alter the nutritional quality of plant products (125), have a negative correlation with digestion coefficient for crude protein (81), and eventually reduce weight gains in poultry and other animals (20,60,65,72,80,118,119).

Results obtained in laboratory seed germination and field

emergence tests showed genotypic variation. Harvest delay did not affect field emergence. However, in genotypes like Seafarer and Tuscola, delay in harvest reduced *in vitro* seed germination. The conflicting results between *in vitro* germination and field emergence can best be explained by the fact that temperature and humidity are rigidly controlled in the germinator (*in vitro* germination). Such conditions favor the germination and development of fungi before seed germination; eventually seed is decayed by fungal growth. In the field, temperature and moisture (humidity) conditions fluctuate considerably and could have been inhibitory to fungi.

Only the Tuscola and Seafarer genotypes showed significant negative correlations between incidence of internal seed-borne fungi and *in vitro* seed germination due to delayed harvest; this agrees with other reports (24,29,34,39,43,44,46,123) for dry bean and (40,104,157) for soybeans. *In vitro* seed germination in several genotypes was not affected by delayed harvest. All of the fungi isolated in the present study appeared to be saprophytes, with no observable negative effect on seed germination. None of the fungi which were reported to reduce *in vitro* soybean seed germination (3) were isolated from bean seed grown in Michigan. Instead, *Alternaria* and *Fusarium spp* which were isolated in this study, were reported to be accompanied by increases in percentage seed germination and decrease in field emergence (40, 157).

Our findings agree with others (28,68) in that black seeded genotypes have general superiority to white seeded types for field emergence. The superiority can be explained as follows:

(a) Black seeded genotypes can adapt much better to adverse

environmental conditions than white seeded cultivars, and consistently possess an advantage over white when planted in cold, wet, or warm soil (28).

(b) Black seeds have greater seed coat dry weight and thickness than white seeds, and these traits are negatively correlated with permeability and rate of osmosis (160). Osmosis through black seed coats may be slowed by a physical barrier of greater cell numbers, by differences in cell density or by some chemical reaction (i.e., phenolic oxidation) unique to colored seeds. Slower absorption of water by colored seeds may permit more uniform swelling of the cotyledons, thereby reducing seed coat and/or cotyledon cracking which are important to germination and early seedling growth (62,63,107,160). Differences in field emergence of two isogenic lines differing only in seed coat color support the importance of seed coat color.

(c) Superiority of black seeded genotypes may be due to resistance to Rhizoctonia root rot (which may have) contributed to differences in emergence. Other lines, however, have reported resistance to this organism and the superior performance of their colored sublines must be attributed to other physiological factors (28). This conclusion is also supported by the difference in field emergence of San-Fernando and Nep-2 both of which were almost equally resistant to Rhizoctonia on the basis of internal seed infection tests.

Seafarer, a genotype with high internal seed infection showed a negative corrleation between delayed harvest and field emergence. This negative effect in some genotypes could be related to the ability of roots of a particular genotype to exude ogranic compounds. Exudation of organic compounds like amino-acids, sugars, and protein from

plant roots and germinating seeds could supply the energy required for a seedling parasite to grow (77,105,109,128,130,155). Seeds of pea varieties most susceptible to damping-off exuded greater amounts of amino-acids and sugars during germination (54,129) than resistant varieties.

Bean genotypes with colored seeds produced stronger and more vigorous seedlings than those with white seeds. This effect probably involves phenolic metabolism of the seedlings. The importance of phenolic compounds in resistance of bean plants to seedling diseases has been reported to be manifested during the early stages of plant development (141). This supports the statement made by Deakin (28) "yield advantages conferred by color seed are largely effective at the early stages of growth and are probably related to superior emergence and seedling vigor."

SUMMARY AND CONCLUSIONS

A two year series of experiments were conducted in the laboratory and green house, Michigan State University, and in the field at the Saginaw, Michigan Bean and Beet Research Farm, and at the Crop and Soil Science Research Farm, East Lansing, Michigan.

Objectives:

(a) to study the effect of genotype and harvest date on incidence of external and internal seed-borne fungi in dry bean (Phaseolus vulgaris L) as affected by delayed harvest;

(b) to determine specific fungus X genotype interactions;

(c) to study the effect of genotype and delayed harvest on in vitro seed germination and field emergence;

(d) to study in the preliminary way the genetics of resistance to internal seed-borne fungi.

Summary of Results:

1. Degree of pod molding increased as time of harvest was delayed after normal maturity.

2. No genotypic variation was observed for the degree of surface infestation of seed by fungi.

3. There is genotypic variation for seed infection by internally seed-borne fungi. San-Fernando and Seafarer were the most and least resistant of the several dry bean genotypes tested, respectively.

4. Delayed harvest after normal maturity does not affect all bean genotypes in the same way. There is increase in internal seed infection in susceptible genotypes such as Seafarer and Tuscola due to increasing harvest delays. Internal seed infection decreased in resistant genotypes like San-Fernando harvested after normal maturity.

5. Eight fungi were isolated from surface-sterilized bean seed such as: species of Alternaria, Rhizoctonia, Fusarium, Penicillium, Cladosporium, Epicoccum, Chaetomium, and Rhizopus. Alternaria and then Rhizoctonia spp were the most frequently isolated fungi.

6. Two specific fungus x genotype interactions were found. Alternaria was isolated most frequently from Tuscola and least frequently from San-Fernando; the reverse was true for Rhizoctonia.

7. Genes affecting seed infection under wet weather conditions display addivity to slightly partial dominance for the resistant response.

8. Genes for resistance are independent of seed coat color. San-Fernando and Nep-2 isogenic except for seed color, showed no significant difference in internal seed infection. F_1 seeds (F_2 germs) of San-Fernando and Tuscola, although black in color, showed higher infection than the resistant parent (San-Fernando).

9. Turrialba #1, a genotype of larger seed size than Seafarer, showed less internal seed infection than Seafarer; seed size may not always be strongly associated with resistance.

10. To obtain high quality seed of susceptible genotypes, seed should be harvested as soon as practical after normal maturity.

11. Black seeded genotypes exhibit better field emergence than white seeded genotypes. Incidence of internal seed-borne fungi and

field emergence were not correlated in all genotypes. Genotypes which exhibited high in vitro seed germination showed very poor field emergence. This discrepancy has to do with intact seed coat and seed coat thickness; genotypes with thick seed coats have higher field emergence. In vitro seed germination may not be a good method to predict field emergence.

12. There is significant genotypic variation for in vitro seed germination and field emergence. Delay in harvest after normal maturity does not affect in vitro seed germination and field emergence the same in all genotypes. Delayed harvest had a negative effect on in vitro seed germination in susceptible genotypes.

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