Effect of Growing Location on Internally-Seedborne Fungi, Seed Germination, and Field Emergence of Pigeon Pea in Puerto Rico[1]

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ABSTRACT

Fungi representing 10 genera were isolated from internal tissues of pigeon pea seeds of four cultivars grown at Isabela, P. R. Seed quality of all cultivars tested from Isabela was low (low percentage seed germination and high incidence of seedborne fungi). The occurrence of fungi from seeds of the cultivar 2B-Bushy, which had poor physical appearance (wrinkled seedcoat and discolored) was higher, and germination was lower, than for seeds of good physical appearance (uniform shape and color).

Seed of cultivar 2B-Bushy produced at Fortuna, P.R. had less internally-seedborne fungi and greater germination in vitro and emergence in the field than seeds produced at Isabela. The occurrence of total internally-seedborne fungi, *Phomopsis* sp., *Lasiodiplodia theobromae*, *Fusarium semitectum*, and *Alternaria tenuissima* was negatively correlated with emergence in the field. The occurrence of *Aspergillus* sp. was not negatively correlated with field emergence.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is a tropically-adapted bush legume which provides a rich source of protein (6). In 1975–1976, the pigeon pea industry contributed $3.3 million to the economy of Puerto Rico (2).

In 1975–1976, one of the limiting factors of pigeon pea production in Puerto Rico was poor seed germination and emergence in the field. The average field emergence of some 4-hectare fields was as low as 28 to 45%. Ellis et al. (3) reported that seed used for planting these fields had an in vitro germination and incidence of internally-seedborne fungi of 60 and 75%, respectively. This suggests that internally-seedborne fungi may play a major role in reducing the quality of pigeon pea seed in Puerto Rico.

Studies on soybean [*Glycine max* (L.) Merr.] (5, 7) and dry bean (*Phaseolus vulgaris* L.) (4) have shown that as the percentage of seeds infected by internally-seedborne fungi such as *Phomopsis* sp., *Fusarium* sp., and *Alternaria* sp. increases, the percentage seed germination and field emergence decreases.

In order to increase the production of pigeon pea in Puerto Rico, a source of high-quality disease-free seed must be made available to growers. Little has been published on the control of internally-seedborne

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microorganisms of pigeon pea and their effect on seed germination and field emergence (1). This study reports on the effect of internally-seedborne fungi on seed quality of pigeon pea, and the effect of growing location in Puerto Rico on seed infection by fungi and seed quality of pigeon pea.

MATERIALS AND METHODS

In order to determine the seed quality of pigeon pea produced at Isabela, P.R., seeds of the cultivars, 2B-Bushy, Line 12, Line 69-68, and Kaki were obtained from cold storage at the Isabela Substation of the Agricultural Experiment Station. All seeds were produced in the 1975-1976 growing season at Isabela. Four hundred seeds of each cultivar were bioassayed for the presence of internally-seedborne fungi and germination in vitro. Seeds were surface disinfected by soaking in a 0.25% sodium hypochlorite solution for 2.5 min, followed by 70% ethanol for 2 min, and finally rinsed in sterile, distilled water. Seeds were then transferred to potato dextrose agar (PDA) in culture plates (four seeds per plate) and incubated at 25° C. After 7 days the occurrence of internally-seedborn fungi and percentage seed germination in vitro were recorded. All internally-seedborne fungi isolated from seed were identified at least to genus. Four replications of 100 seeds/cultivar which were not surface disinfected, were planted in a sandbench at the Isabela substation. Seedling emergence in sand was recorded after 10 days.

In a second experiment, 400 seeds of cultivar 2B-Bushy, which had good physical appearance (without wrinkles or cracks in the seedcoat and uniform in color), were selected. An additional 400 seeds, which had poor physical appearance (cracked or wrinkled seedcoats and discolored), were also selected. Both sets of seeds were surface disinfected and bioassayed as previously described.

In a third experiment, seed samples of cultivar 2B-Bushy were collected from seed production fields located at the Fortuna and Isabela substations. The following fields with their location, number of hectares planted, date of planting, and date of harvest, respectively, were sampled: 1) Isabela I, 2.43, August 13, 1976, January 4, 1977; 2) Isabela II, 3.24, August 16, 1976, January 11, 1977; 3) Fortuna I, 2.43, October 13, 1976, February 7, 1977; and 4) Fortuna II, 5.67, November 10, 1976, March 15, 1977. After harvest, four samples of 400 seeds were randomly collected from each field. Two hundred seeds from each sample were surface disinfected and bioassayed as previously described. The remaining 200 seeds from each sample were planted in the field at the Isabela substation. Emergence in the field was recorded after 15 days.

The following weather data were collected at the Isabela and Fortuna substations: maximum daily temperature, minimum daily temperature; rainfall and pan evaporation.

Analysis of variance was calculated for percentage emergence in the field, germination on PDA, total internally-seedborne fungi and the recovery of fungi by genera for seeds from all fields sampled. Correlation coefficients were calculated between percentage emergence in the field, total internally-seedborne fungi, and occurrence of fungi by genera.

RESULTS

The following internally-seedborne fungi were isolated from the seeds bioassayed in this study: *Phomopsis* sp.; *Lasiodiplodia theobromae*; *Fusarium semitectum*; *Alternaria tenuissima*; *Aspergillus* sp.; *Nigrospora* sp., *Penicillium* sp.; *Macrophomina* sp.; *Cladosporium* sp. and *Rhizopus* sp. The seeds of all four cultivars which were obtained from cold storage at Isabela were of extremely poor quality. The percentage germination in vitro and emergence in sand did not exceed 60% for any cultivar tested (table 1). The percentage of seeds infected by fungi was not below 65% for all cultivars. *Phomopsis* sp. and *L. theobromae* were almost consistently isolated from dead (nongerminated) seeds. *F. semitectum* was frequently isolated from dead seeds and *A. tenuissima* was most frequently isolated from germinated seeds.

Seeds of the cultivar 2B-Bushy which had good physical appearance had a mean percentage germination in vitro of 93.7% and 64% of the seeds tested germinated and were free of internally-seedborne fungi (table 2). The mean percentage germination in vitro and seeds which germinated free of fungi for seeds of poor physical appearance were 34.3 and 6%, respectively. The isolation of *Phomopsis* sp., *L. theobromae*, *F. semitectum* and *A. tenuissima* was more frequent from seeds of poor physical appearance than from seeds of good physical appearance (table 2).

The mean percentage field emergence and germination in vitro was significantly lower for seeds from both fields produced at Isabela than for seeds produced at Fortuna (table 3). Seed from the second planting at Isabela had significantly greater emergence in the field and germination in PDA than seeds from the first planting at Isabela. The occurrence of total internally-seedborne fungi, *Phomopsis* sp., *L. theobromae*, *F. semitectum*, and *A. tenuissima* was significantly higher for seeds from Isabela than for seeds from Fortuna. Seeds from the first planting at Isabela had significantly more of the above mentioned fungi than seeds from the second planting at Isabela (table 3). Seed from the first planting at Isabela had significantly less *A. tenuissima* than seed from the second planting at Isabela. *Phomopsis* sp. and *L. theobromae* were not isolated from seeds produced at Fortuna. Seeds from the first planting at Fortuna had significantly more *Aspergillus* sp. than seeds from the second planting (table 3). *Aspergillus* sp. was almost constantly isolated from germinated seeds. When field emergence was correlated with the incidence of total internally-seedborne fungi, *Phomopsis* sp., *L. theobromae*, *F. sem-*

TABLE 1.—*Percentage germination in vitro (PDA), emergence in sand, incidence of total internally-seedborne fungi, and occurrence of fungi by genera for seeds of four cultivars of pigeon pea produced at Isabela, P.R., 1975-1976*[1]

Cultivar	Germination in vitro	Emergence in sand	Total fungi	Occurrence of fungi by genera				Miscellaneous fungi
				Phomopsis	*Lasiodiplodia*	*Fusarium*	*Alternaria*	
2B-Bushy	36	38	85	22	21	17	20	5
Line 12	47	51	71	15	19	12	22	3
69-68	52	55	69	13	17	13	23	3
Kaki	55	60	65	10	15	15	19	6

[1] All figures based on 400 seeds/cultivar.

TABLE 2.—*Percentage germination in vitro (PDA), seed which germinated and were free of fungi, incidence of total internally-seedborne fungi, and occurrence of fungi by genera for seeds of the cultivar 2B-Bushy of good and poor physical appearance*[1]

Physical appearance	Germinated free of fungi	Germination in vitro	Total fungi	Occurrence of fungi by genera				Miscellaneous fungi
				Phomopsis	*Lasiodiplodia*	*Fusarium*	*Alternaria*	
Good	64	94	21	3	3	6	7	3
Poor	6	34	93	23	27	17	21	4

[1] All figures based on 400 seeds/seed classification.

TABLE 3.—*Percentage emergence in the field, germination in vitro (PDA), incidence of total internally-seedborne fungi, and occurrence of fungi by genera from seeds of cultivar 2B-Bushy produced at Isabela and Fortuna, P. R., 1976-1977*[1]

Location and date planted	Field emergence	Germination in vitro	Total fungi	Occurrence of fungi by genera					Miscellaneous fungi
				Phomopsis	*Lasiodiplodia*	*Fusarium*	*Aspergillus*	*Alternaria*	
Isabela I	41.0	49.7	73.8	10.8	18.8	19.0	0.2	11.7	12.1
Isabela II	63.0	75.3	35.0	.8	6.5	4.3	1.5	17.0	10.0
Fortuna I	90.0	90.3	23.8	0	0	2.0	17.0	3.0	1.0
Fortuna II	95.0	97.3	10.5	0	0	2.5	3.0	1.7	3.1
LSD .05	3.25	13.85	6.53	1.92	4.26	4.40	4.17	4.60	3.10
LSD .01	4.68	19.89	9.42	2.75	6.14	6.31	5.98	6.60	4.88

[1] All figures based on four replications of 200 seeds/growing location and date of planting.

itectum, *A. tenuissima*, and *Aspergillus* sp., the correlation coefficients were: −.95; −.91; −.93; −.86; −.75 and +.11, respectively.

The mean daily temperatures at the Isabela and Fortuna substations for the time period in which the crops were in the field were about the same (table 4). However, rainfall was considerably less at the Fortuna substation, especially for the time period when the fields were maturing. Pan evaporation was higher at Fortuna than at Isabela.

DISCUSSION

The results of this study indicate that internally-seedborne fungi play a major role in reducing seed quality (percentage germination and field emergence) of pigeon pea in Puerto Rico. The occurrence of total internally-seedborne fungi, *Phomopsis* sp., *L. theobromae*, *F. semitectum*, and *A. tenuissima* was negatively correlated with emergence in the field. As seed infection by these fungi increased the percentage field emergence decreased. In addition, these fungi appeared to affect the physical appearance of the seed. Seeds which were wrinkled and discolored had a much higher percentage infection by fungi than seeds which had good physical appearance. Good physical appearance (uniformity in shape, color, and size) appears to be an important characteristic of high quality pigeon pea seed.

TABLE 4.—*Climatological data from the Fortuna and Isabela substations, 1976-1977*

Substation	Month	Air temperature, °C			Rainfall	Pan evaporation
		Mean maximum	Mean minimum	Mean		
					cm	*cm*
Isabela	September	30.1	20.3	25.2	8.71	13.23
	October	28.7	20.8	24.6	6.50	11.81
	November	29.3	18.5	23.9	9.47	13.54
	December	28.1	16.6	22.4	12.70	10.59
	January	27.5	17.8	22.7	14.35	10.16
	Mean	28.8	18.8	23.7	10.34	11.86
Fortuna	November	31.2	21.2	26.2	1.24	13.31
	December	30.1	19.8	24.9	2.03	13.99
	January	29.5	18.6	24.1	.68	14.10
	February	30.0	19.7	24.8	.81	15.32
	March	30.4	18.9	24.7	1.52	20.50
	Mean	30.2	19.6	24.9	1.24	15.44

The seed quality of all cultivars produced at Isabela was extremely poor (low percentage germination and high percentage of seeds infected by fungi). This indicates that the quality of seed produced and used by farmers in the region of Isabela is poor. Seeds produced at Fortuna had excellent seed quality (high percentage germination and low percentage of seeds infected by fungi). The differences in the quality of seeds produced at Fortuna and Isabela are probably due to the differences in climate between the two regions. Humidity is probably the most important factor. Due to the low amount of rainfall and high amount of pan evaporation, the climatic conditions at Fortuna were much drier than at Isabela. Fungi usually require water for growth, reproduction, spore germination, and penetration of plant tissues.

The results of this study indicate that the selection and use of specific regions for production of pigeon pea seed in Puerto Rico could greatly increase seed quality. When seed production areas have been identified, they could be used to produce high quality seeds for other crops such as field bean, soybean, sorghum, and cowpea.

RESUMEN

Hongos de 10 géneros se aislaron del tejido interno de las semillas de cuatro cultivares del gandul (*Cajanus cajan* (L) Millsp.) sembrados en las subestaciones experimentales de Fortuna e Isabela, Puerto Rico. La calidad de las semillas de todos los cultivares sembrados en Isabela fue baja (un bajo porcentaje de germinación y una alta incidencia de hongos). La presencia de hongos en las semillas del cultivar 2B-Bushy, de pobre apariencia (testa arrugada y descolorida), era más abundante, y la germinación más pobre que en las semillas de buena apariencia (forma y color uniformes).

En el tejido interno de las semillas del cultivar 2B-Bushy cosechado en Fortuna había menos hongos. Estas germinaron mucho mejor, tanto *in vitro* como en el campo, que las cosechadas en Isabela.

La presencia en el tejido interno de los hongos *Phomopsis* sp., *Lasiodiplodia theobromae*, *Fusarium semitectum* y *Alternaria tenuissima* estuvo correlacionada negativamente con la germinación en el campo, pero no así la de *Aspergillus* sp.

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