PATHOLOGIC REACTION OF GARDEN PEA (*Pisum sativum* L) CULTIVARS/ ADVANCED BREEDING LINES TO *Fusarium oxysporum f sp pisi* (Linford) Synder and Hansen[1]

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ABSTRACT

Pot experiments were carried out under greenhouse conditions to evaluate the resistance of eight garden pea cultivars/ advanced breeding lines to *Fusarium oxysporum* f. sp. *pisi* (FOP). The effect of FOP inoculation on plant height, fresh and dry weights of shoots and roots and number and weight of pods were assessed 60 days after planting. Stems were examined for necrotic lesion scores.

Based on the necrotic lesion score and pathologic reaction of plants at 60 days after inoculation, Betag, CGP 110 and 154 were rated as resistant; CGP 59, 11, and 34 as intermediate; and CLG and CGP 13 as susceptible to *Fusarium* wilt.

Key Words: resistance, inoculation, *Fusarium oxysporum* f sp *pisi*, plant parameters, necrotic lesion score

INTRODUCTION

Garden pea (*Pisum sativum* L.) locally known as "Chinese pea", "sweet pea", or "sitsaro" is one of the high-value leguminous vegetables in the Philippines. It is grown for its edible pods and seeds. It grows well in the temperate zone of Benguet as a cool-season crop throughout the year (PCARRD, 2008). It is a source of protein, amino acids, sugars, carbohydrates, vitamins A and C, calcium, phosphorus and a small quantity of iron (DAFF, 2011). The Cordillera Administrative Region is the major producer of garden pea having a national share of 94.5%. In garden pea production, the high incidence of plant pest like leaf miner and pathogens like *Fusarium* wilt, powdery mildew and plant-parasitic nematodes, had reduced the harvested area by 1.4% from 1,697 hectares in 2005 down by 1, 674 hectares in 2006 (PCARRD, 2008).

The utilization of resistant crops is one of the most effective components of integrated pest management and the inclusion of this property ensures increased crop yield in the presence of plant pathogens (Khan, 2008). It is also an economical and environmentally safe method in the control of soil-borne pathogens (Tariq, 2008). Host plant resistance (HPR) is an inherited property that enables a plant to avoid, tolerate, or recover from injury by pest populations (Yang, 2008). It is also a plant characteristic which influences the ultimate degree of damage done by the pest (Painter, 1951) or reduces the probability of successful utilization of the plant by the pest (Beck, 1965). Therefore, the growing of resistant varieties against the target fungal species demands correct identity of fungi existing in the area (Khan, 2008).

Garden pea wilt caused by *Fusarium oxysporum* can be devastating. It is equally as important as the disease of tomato, tobacco, legumes, cucurbits, sweet potatoes, banana and other herbaceous plants. The pathogen invades the plant's roots with its germ tube or mycelium.

Once inside the plant, the mycelium grows through the root cortex reaching the xylem as it advances upwards toward the stem and crown of the plant. Due to the growth of the fungus within the plant's vascular tissues, the water supply is greatly affected. Lack of water induces the leaves' stomata to close, the leaves

wilt, and the plant eventually dies (Agrios, 1988).

This study was undertaken to evaluate the pathologic reaction of eight garden pea cultivars/ advanced breeding lines to *Fusarium* wilt under greenhouse conditions.

MATERIALS AND METHODS

Experimental design

The Completely Randomized Design (CRD) in a factorial experiment was employed with random effect model wherein four test plants in an experimental group were randomly selected for statistical analysis.

Potting Mix preparation

The soil with a proportion of 1:1 garden soil and sand was heat-sterilized in an autoclave at 15 psi for 1 hour. The soil was potted into 1.5 liters capacity PVC pots.

Fusarium oxyposrum f *sp pisi* (FOP)

A pure culture of FOP was standardized by the addition of 10 mL sterile distilled water. Using a micropipette, a 0.1 aliquot was drawn and placed into the wells of the hemocytometer.

The microconidia and macroconidia of the fungi were counted using Nikon™ YS100 under 400x magnification and were calculated accordingly. A standardized inoculum of FOP suspension (1 X 106 cfu/mL) was inoculated into heat-sterilized potted soil.

Garden pea cultivars

Healthy seeds of eight garden pea varieties/ advanced breeding lines, namely Chinese Green Pea (CGP) 59, CGP 11, CGP 110, CGP 34, CGP 154, CGP 13 and two check varieties (Betag and Chinese Light Green (CLG)) were obtained from the Highland Crops Research Station, Institute of Plant Breeding, Benguet State University, La Trinidad, Benguet.

The seeds were disinfected with 1% NaOCl for 10 minutes and were rinsed thrice with sterile distilled water. The seeds were blot dried in sterile tissue paper prior to planting.

Seeds were seeded directly in PVC pots with soils previously inoculated with standardized FOP suspension. The test plants were maintained at 60 days for assessment of FOP infection. The necessary plant management practices such as fertilizer application and specific control measures of associated insect pests and pathogens in garden pea except *Fusarium* wilt were employed under greenhouse conditions.

Assessment of plant growth and yield

Plant height, fresh and dry weights of shoots and roots, and the number and weight of pods were obtained to assess resistance and susceptibility to FOP.

Damage analysis

The pathologic reaction was assessed using the necrotic lesion scoring of Speijer and De Waele (1997) (Coyne *et al*., 2007) and the damage analysis by Van Schoonhoven and Pastor-Corales (1994). A pictorial scale of 0% as clean (without necrotic lesion) to 100% as with the highest degree of necrotic lesion was used to measure FOP damage.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and multiple comparisons using Duncan's Multiple Range Test (DMRT) where differences among treatments were separated at 1% and 5% levels of significance.

For variables measured using counts, the square root transformation was employed and for data in percent, the arcsin transformation was used to normalize variances.

RESULTS AND DISCUSSION

Effect of FOP inoculation on plant growth and yield

Inoculation of garden pea seeds with FOP has significantly affected plant height, fresh and dry weights of shoots and roots, and the number and weight of pods at 60 days after inoculation. There was no significant effect of FOP inoculation on dry weight of roots (Figure 1).

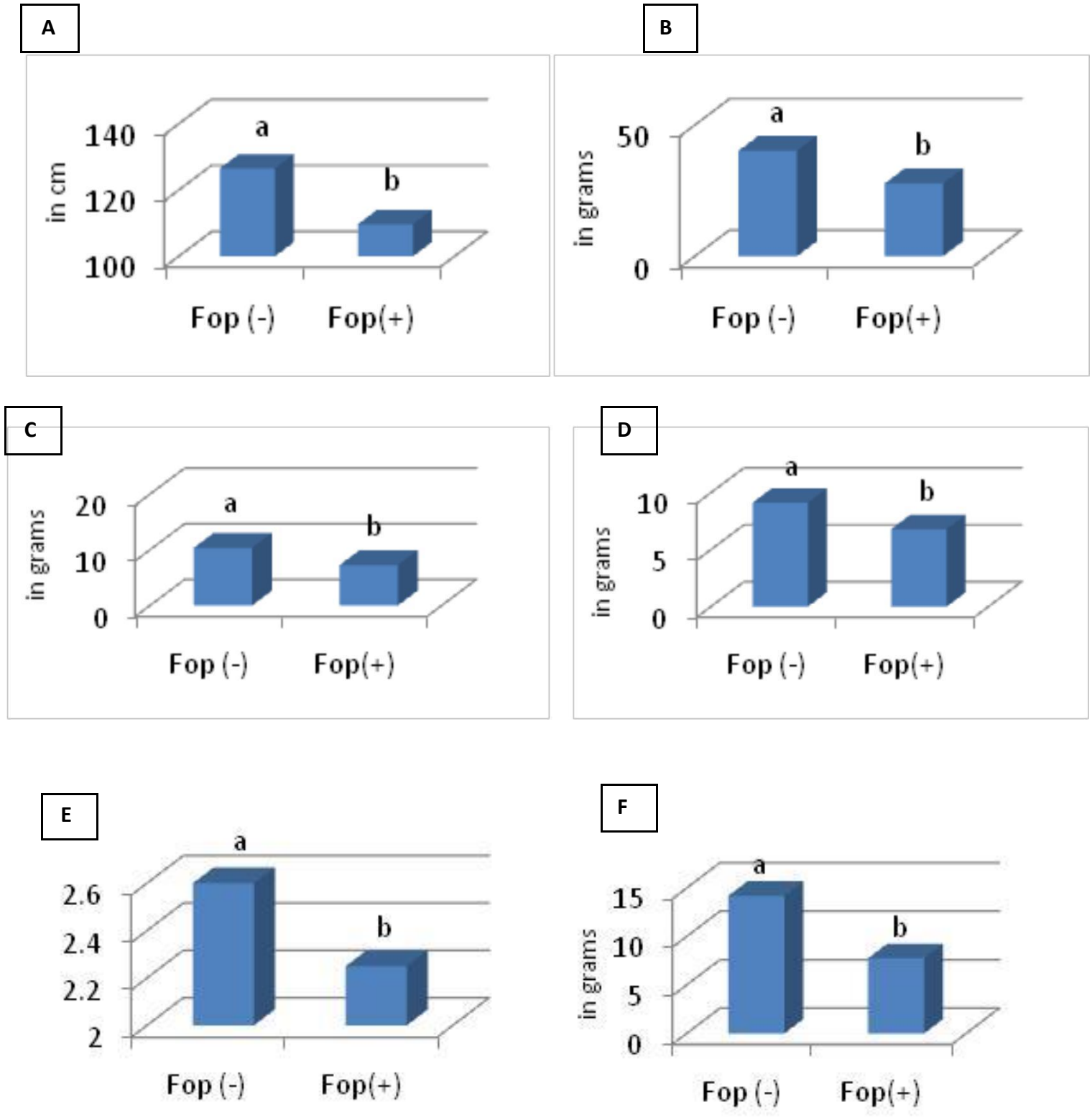

Figure 1. Main effect of FOP inoculation on a. plant height (cm), b. fresh weight of shoots (g), c.dry weight of shoots (g), d. fresh weight of roots (g), e. number of pods, and f. weight of pods (g)

Moreover, there were significant differences ($P < 0.05$) observed in plant height, fresh and dry weights of shoots, fresh weight of roots and in the number of pods between the inoculated (FOP+) and uninoculated (FOP-) plants among the eight cultivars/ advanced breeding lines. A significant reduction in plant height was observed in CGP 11 (21.9%), CGP 110 (16.2%), CGP 154 (23.1%), CGP 13 (21.1%), and CLG (32.6%). On the contrary, inoculated Betag plants showed a significant increase in plant height of 22.2% compared with uninoculated plants. A significant reduction in the fresh weight of shoots was observed in CGP 34 (48.52%) while CGP 59 (48%) and Betag (46.1%) had a significant reduction in dry weight of shoots. Moreover, CLG had significant reduction on fresh weight of roots of 68.6%. Apparently, there was no significant reduction or increase observed in dry weight of roots and in the number of pods. Significant reduction in the weight of pods was observed in Betag (57%). Seven out of eight varieties/ advanced breeding lines inoculated plants showed a significant reduction in plant height ranging from 5.0% - 32.6% (Table 1).

Table 1. Effects of *F. oxysporum* f. sp. *pisi* on the growth of eight garden pea varieties/advanced breeding lines

	PLANT PARAMETERS											
Var/ ABL	**Plant height (cm)**			**Fresh shoot weight (g)**			**Dry shoot weight (g)**			**Fresh root weight (g)**		
	Fop-	**Fop +**	**%change**	**Fop-**	**Fop+**	**%change**	**Fop-**	**Fop+**	**%change**	**Fop-**	**Fop +**	**%change**
1. 59	115.25^{bcde}	109.50^{de}	(-) 5.0	30.65^{cdef}	30.66^{cdef}	(+)0.03	13.92^{a}	7.24^{c}	(-)48.0	7.38^{bcde}	8.29^{bcd}	(-)12.3
2. 11	130.93^{bc}	102.25^{e}	(-)21.9	33.12^{cde}	25.46^{ef}	(-)23.13	9.21^{bc}	8.47^{bc}	(-)8.0	7.33^{bcde}	5.55^{de}	(-)24.3
3. 110	130.93^{bc}	109.75^{de}	(-)16.2	33.12^{cde}	23.70^{ef}	(-)28.44	9.21^{bc}	6.14^{c}	(-)33.3	7.33^{bcde}	7.63^{bcde}	(+)4.1
4. 34	129.25^{bcd}	114.50^{bcde}	(-)11.4	43.69^{b}	22.49^{f}	(-)48.52	10.74^{b}	8.15^{bc}	(-)24.1	6.08^{cde}	8.32^{bcd}	(+)36.8
5. 154	130.93^{bc}	100.75^{e}	(-)23.1	33.12^{cde}	27.58^{ef}	(-)16.73	9.21^{bc}	7.62^{bc}	(-)17.3	7.33^{bcde}	7.13^{bcde}	(-)2.7
6. 13	130.93^{bc}	103.25^{e}	(-)21.1	33.12^{cde}	26.67^{ef}	(-)19.47	8.78^{bc}	6.51^{c}	(-)25.9	7.33^{bcde}	4.83^{de}	(-)34.1
7.Betag	133.60^{a}	163.25^{a}	(+)22.2	37.69^{bcd}	40.18^{b}	(+)6.61	14.24^{a}	7.68^{bc}	(-)46.1	11.22^{b}	9.49^{bcd}	(-)15.4
8. CLG	110.50^{cde}	74.50^{g}	(-)32.6	29.68^{def}	25.09^{ef}	(-)15.46	8.34^{bc}	6.35^{c}	(-)23.9	10.42^{bc}	3.27^{e}	(-)68.6

Var/ ABL	**Dry root weight (g)**			**No. of pods**			**Weight of pods (g)**		
	Fop-	**Fop+**	**%change**	**Fop-**	**Fop+**	**%change**	**Fop-**	**Fop +**	**%change**
1. 59	0.28^{b}	0.24^{b}	(-)14.3	2.86^{ab}	2.03^{bc}	(-)29.0	9.67^{bc}	4.75^{c}	(-)50.9
2. 11	0.34^{b}	0.24^{b}	(-)29.4	2.57^{abc}	1.67^{cd}	(-)35.0	13.00^{bc}	4.25^{c}	(-)67.3
3. 110	0.34^{b}	0.20^{b}	(-)41.2	2.57^{abc}	2.85^{ab}	(+)10.9	13.00^{bc}	11.25^{bc}	(-)13.5
4. 34	0.39^{b}	0.20^{b}	(-)48.7	2.68^{ab}	2.06^{bc}	(-)23.1	14.75^{b}	10.00^{bc}	(-)32.2
5. 154	0.34^{b}	0.22^{b}	(-)35.3	2.57^{abc}	2.17^{bc}	(-)15.6	13.00^{bc}	8.50^{bc}	(-)34.6
6. 13	0.34^{b}	0.20^{b}	(-)41.2	2.57^{abc}	2.34^{bc}	(-)8.9	10.00^{c}	5.50^{c}	(-)45.0
7.Betag	0.63^{b}	0.37^{b}	(-)41.3	3.36^{a}	2.80^{ab}	(-)16.7	26.75^{a}	11.50^{bc}	(-)57.0
8. CLG	0.36^{b}	0.13^{b}	(-)63.9	2.54^{abc}	1.99^{bc}	(-)21.7	11.67^{bc}	6.50^{bc}	(-)44.3

Data are means of 4 replicates and transformed to the √x+.5 prior to statistical analysis. Means with the same letter in a column do not differ significantly at P>0.05 according to Bonferroni and Duncan's Multiple Range Test (DMRT).

Effect of FOP inoculation on fungal parameters

Wilt symptom. The appearance of wilt symptom was first observed in cultivar/ advanced breeding lines of CGP 13, CGP 154, CLG, CGP 34 and CGP 110 at 44 days. Although insignificant, Betag, CGP 11 and CGP 59 followed at 45-46 days (Table 2).

Necrotic lesion. The CGP 110 (4%), CGP 154 (9%) and Betag (4%) cultivars had the lowest necrotic lesion mean scores rated as resistant to FOP infection whereas CGP 13 (30%) and CLG (40%) had the respective highest mean percentages rated as susceptible. On the other hand, CGP 59, 11 and 34 were rated with respective scores of 24%, 28%, and 15% as intermediate (Table 2). Vascular necrosis of representative garden pea plants is shown in Figure 3.

Figure 2. Garden pea plants at 30 days (left) and 60 days (right) after inoculation of *F.oxysporum* f.sp. *pisi.*

Table 2. Mean number of days to the first appearance of wilt symptom, necrotic lesion scores, rating and pathologic reaction of eight garden pea cultivars

Var/ ABL	No. of days of the first appearance of wilt symptom	Necrotic lesion score[1](in %)	Rating[2]	Pathologic reaction (PR)[2]
1. 59	46.70[a]	24[ab]	4	Intermediate
2. 11	46.30[a]	28[ab]	4	Intermediate
3. 110	44.80[a]	4[cd]	1	Resistant
4. 34	44.70[a]	15[bc]	4	Intermediate
5. 154	44.00[a]	9[c]	2	Resistant
6. 13	44.00[a]	30[ab]	7	Susceptible
7.Betag	45.20[a]	4[cd]	1	Resistant
8. CLG	44.10[a]	40[a]	8	Susceptible

Data are means of 4 replicates and transformed to arcsin prior to statistical analysis. Means with the same letter in a column do not differ significantly at P>0.05 according to Bonferroni and Duncan's Multiple Range Test (DMRT). [1] Necrotic lesion score was adapted from Speijer and De Waele (1997) [2] Rating and pathologic reaction is based from general scale in evaluation of bean germplasm reaction to fungal and bacterial pathogens by Van Schoonhoven, A. and Pastor-Corrales, M.A. (1994) Range of rating: 1-3: Resistant- No visible symptoms or very light symptoms, 4-6: Intermediate- visible and conspicuous symptoms resulting only in limited economic damage, 7-9: susceptible- severe to very severe symptoms causing considerable yield losses or plant death.

Figure 3. Vascular necrosis of representative stems of garden pea. TOP: Betag variety at 5% (resistant) MIDDLE: CGP 59 at 25% (intermediate) BOTTOM: CLG at 100% (susceptible)

The eight garden pea cultivars/advanced breeding lines varied in their response to FOP inoculation. Cultivar Betag and variety CLG showed significant results in plant height and fresh weight of shoots and roots. At 60 days after inoculation, Cultivar Betag was rated as resistant based on its necrotic lesion score and pathologic reaction to FOP.

It was observed that the high plant height of Betag corresponds to its high fresh shoot weight in inoculated groups. This result is attained to its high fresh root weight which contributed much on plant growth. Inspite of inoculation, Betag had the highest fresh root weight which enabled it to overcome the development of the wilt disease. This also explains the low necrotic lesion score in the vascular region of the said garden pea cultivar.

Breedling lines CGP 110 and 154 were equally rated as resistant based on necrotic lesion score. However, plant height and fresh weight of shoots of CGP 110 and 154 were attributed to pea enation mosaic virus which infected the plants. This is an aphid-transmitted virus which infected the garden pea plants that developed mosaic and chlorotic vein flecking which appeared as translucent windows and veinal enations as blister-like outgrowths.

It has been observed that it was accompanied by a downward leaf rolling. The virus aggravated the stunting of CGP 110 and 154. It was noted that the

plants recovered due to constant water and pesticidal sprays done to reduce aphid infestation. On the other hand, cultivar CLG cultivar had a significant reduction in plant height and fresh weight of shoots and roots upon FOP inoculation. This is attributed to its high necrotic lesion score which eventually led to poor plant growth.

The variety CLG was observed to be the first to have wilted leaves. This symptom is characterized by dry and brittle leaves accompanied by chlorosis or leaf yellowing. These symptoms could be responsible for its low fresh shoot weight. The number and weight of pods of cultivar CLG were comparable to those of the resistant variety, Betag. This comparable high yield of CLG indicates its tolerance to *Fusarium* wilt. This conforms to the study of Huang et al., (2005). In spite of being rated as susceptible, as based on the necrotic lesion score, CLG could be in the level of moderate susceptibility that account for higher plant yield.

CONCLUSIONS AND RECOMMENDATIONS

Based on host response index, cultivar Betag, CGP 110 and 154 are resistant; breeding lines CGP 59, 11, and 34 were intermediate and variety CLG and breeding line CGP 13 are susceptible to FOP infection in garden pea.

Further screening and characterization of other locally available varieties that could be utilized as commercial sources of resistance to FOP is essential for it to become a component of a sustainable fungal management system.

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