Prevalence of *Ascochyta pinodes* on Field Pea Seed Produced in Central Ethiopia and its Relation to Seedling Infection

Dereje Gorfu[1] and Somsiri Sangchote[2]
[1]Holetta Agricultural Research Center, P.O.Box 2003 Addis Ababa, Ethiopia and
[2]Kasetsart University, Department of Plant Pathology, Bangkok 10903, Thailand

Abstract

Ninety-four seed samples of field pea (*Pisum sativum*) from major production areas of central Ethiopia were assessed for prevalence of *Ascochyta pinodes* using blotter method in the laboratory and seedling symptom test in the greenhouse. Consequently, percentage seed infection with *A. pinodes* varied from location to location and from sample to sample. Out of tested samples, 57.4% were infected with *A. pinodes* where some were severely infected as high as 23%. Highest mean prevalence of 8.6% was recorded from Ziquala followed by Minjar with 6.7%. Low seed infection was recorded from Ambo (0.7%), Chelia (1.1%) and Guha Tsiyon (1.0%). All heavily infected seed lots (>15%) were from Ada, Ziquala, Minjar and Kulumsa comprising 7.4 % of the total seed lots while all seed lots from Ambo, Chelia and Goha Tsiyon had infection <5%. No production area was found free of this pathogen. Though blotter method yielded slightly higher incidence, strong and positive linear relationship was obtained between seedling symptom test (dependent) and blotter method (independent variable) explained by equation $Y = 0.8429X - 0.2611$ ($df = 92$; $R^2 = 0.9408^{**}$). *A. pinodes* was found to be transmitted from seed to seedling at the rate of about 84% being highly seed transmissible pathogen. Hence, either method could be used for assessing this pathogen. Most seed lots had germination percentage over 96%, though seeds from Sendafa had lower germination percentage (92.2%). There was no correlation between seed infection by *A. pinodes* and germination percentage of the seeds.

Introduction

Field pea (*Pisum sativum* L.) is important legume widely grown in Ethiopia and central Ethiopia is among major producing regions of the country. The crop is grown during cool and wet period of the year from June to November. The crop is usually grown in rotation with barley, wheat and tef (Gebre *et al.*, 1989), and occasionally in mixture with faba bean (Gorfu, 1999). Sowing is done in late June to early July and harvested during October and November depending on the local climatic conditions. Productivity of this crop is often threatened by Ascochyta blight.

Ascochyta pinodes Jones [teleomorph = *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.] causes ascochyta blight of field pea and this pathogen is the most destructive component of this blight disease complex in Ethiopia (Gorfu and Beshir, 1994) causing up to 53% yield loss under severe conditions (Gorfu, 2000). *A. pinodes* is considered as important seedborne pathogen in many countries (Corbiere *et al.*, 1994; Bretag *et al.*,

1995; Michall *et al.*, 1998) but little is known about the seedborne nature under Ethiopian conditions where the crop is widely grown and the disease economically important.

In Ethiopia, almost all farmers save their own seed for planting (Mariam, 1992), which increases concern on the seed health status of seeds used for planting, as it is not produced with necessary care. Infected seeds are among important means by which this pathogen survives between seasons and also spread into disease free areas (Bretag *et al.*, 1995; Abdel-EL-Rehim *et al.*, 1997; Michall *et al.*, 1998).

Relatively little is known about seedborne mycoflora of field pea grown in Ethiopia. Gorfu and Sangchote (2004) for the first time reported fungi associated with field pea seeds and high incidence of *A. pinodes* among seedborne pathogens after assessing five commercial field pea seed lots collected in 2002 from central Ethiopia. However, no systematic survey was done to clarify the prevalence and seedborne nature of *A. pinodes* in the major production areas of Ethiopia.

This paper reports result of a survey conducted to determine the prevalence of *A. pinodes* in farmer-saved seeds grown in central Ethiopia where field pea is widely grown and ascochyta blight is a major disease.

Materials and Methods

The seed samples

Ninety-four seed samples of field pea (*Pisum sativum* L.) were collected from farmer-saved reserve seeds for planting in subsequent crop season. The collection was done in the major field pea producing areas (Table 1) of central Ethiopia characterized by different altitudes and agroclimatic conditions. Each collection area was indicated by reference location in Figure 1 namely Abo church, Ambo, Arerti, Asela, Debre Zeit, Fiche, Gedo, Goha Tsion, Inewari, Holetta and Sendafa. Samples were randomly collected at every 7 to 10 km in each area. Among samples, whenever possible, one popular farmers' cultivar and a released variety were considered from each collection area. All the samples were harvested from crops grown in October and November of the previous years and collected in March and April of 2003 for seed assessment. All seed samples were grown by subsistence farmers and were reserved for planting and stored in sacks and several kinds of local storage structures. The temperature in these places ranged from 6 to 30^{O}C during storage from November to March, and when the seeds were sampled for analysis. Uniform and representative seed lots were obtained by proper sampling of the seed lots as recommended by ISTA (2003).

Detection and identification of *A. pinodes*

Prevalence of seedborne *A. pinodes* in field pea seed from eleven major production areas in central Ethiopia was studied using blotter method in laboratory and seedling symptom test in greenhouse. In the blotter method, 400 seeds from each seed lot were tested in Noble Germinator with two layers of moistened and sterilized blotter paper and water at the bottom of the seed set that was linked with paper strip to replenish moisture constantly to the seedlings. From each seed lot, 90 seeds were placed in each Noble Germinator and the remaining in another additional set. These sets were incubated at a temperature of 23 ± 2^{O}C under alternating light and dark period of 12 hours. The light spectrum includes near ultra-violate light (NUV) produced by black fluorescent lamp of 40W (Philips black fluorescent lamp).

Table 1. Survey area, reference location, altitude, distance from Addis Ababa and the radius of sampling of field pea seeds collected to study the prevalence of *Ascochyta pinodes* in central Ethiopia.

Area surveyed	Reference location	Altitude (meters)	Distance from Addis (km)	Survey Radius (km)
Ada	Debre Zeit	1950 – 2100	45	20
Ambo	Ambo	2000 – 2500	125	30
Chelia	Gedo	2300 – 2850	175	20
Debre Tsige	Fiche	2250 – 2750	115	30
Goha Tsiyon	Goha Tsiyon	2250 – 2400	180	15
Inewari	Inewari	2250 – 2800	185	35
Kulumsa	Asela	2200 – 2300	175	35
Minjar	Arerti	1980 – 2200	100	10
Sendafa	Sendafa	2300 – 2600	40	25
Welmera	Holetta	2300 – 2500	44	20
Ziquala	Abo church	1960 – 2250	68	10

After 12 days, seeds were assessed for presence of *A. pinodes* under stereomicroscope directly or compound microscope after slide preparation. The incidence of *A. pinodes* was recorded from each set. Identification of the fungus was performed under microscope by comparing with descriptions made by Lawyer (1984). The blotter method was found to be the best and suitable for detecting *A. pinodes* in the previous study (Gorfu and Sangchote, 2004).

In the seedling symptom test, each seed lot was planted in sterilized soil collected from a field where field pea is usually grown in rotation with cereals. The soil was a red clay soil (Nitsols) with a pH of 5.6 and low fertility. Hence, soil for this study was sampled after the field was prepared for planting and the necessary inorganic fertilizer was added according to research recommendations. The soil was moistened and sterilized with soil sterilizer three times every other day. Four hundred seeds from each seed lot were planted in plastic pots at a depth of about 3 cm. Ten seeds were sown in each pot and watered regularly throughout the experimentation period. After 21 days, plants were separated from soil and washed. Ascochyta infection shows conspicuous symptoms on seedlings and hence assessed according to Gorfu (2004). Wherever necessary, isolation was done on potato dextrose agar (PDA) to confirm identification. The greenhouse maintained fluctuation of temperature between a minimum temperature of 11 ^{o}C in nights and maximum temperature of 26^{o}C in daytimes.

The quantitative data collected was subjected to statistical analysis using SAS procedures (Cody and Smith, 1997).

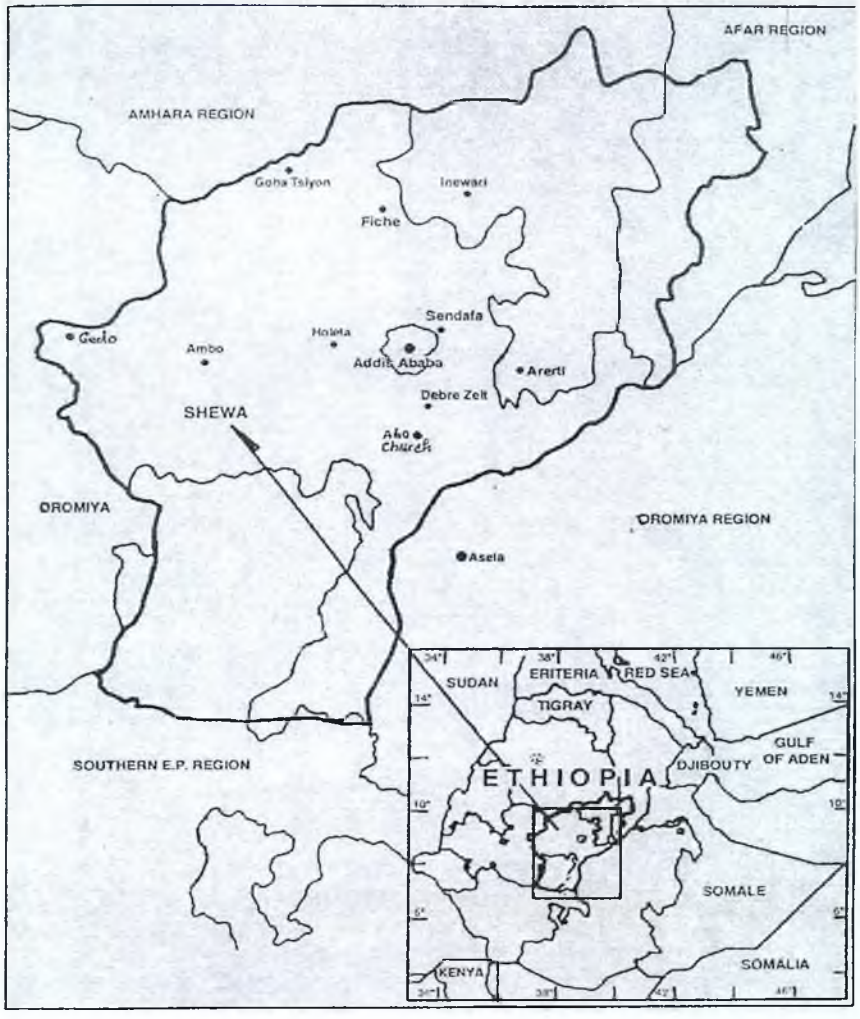

Figure 1 Survey areas of farmer seed samples of field pea in central Ethiopia

Results

There was considerable variation between areas surveyed in both percentage seed lots infected and the degree of seed infection by *A. pinodes* in each seed lot (Table 2). Among 94 seed samples tested, 57.4% of them was found to be infected with *A. pinodes* where some of them being severely infected as high as 23%. Percentage seed lots infected with *A. pinodes* varied from 30% around Ambo to 75% around Ziquala. Ambo is in the west while Ziquala is in eastern part of the survey area (Figure 1). Highest percentage of seed samples (36.2%) showed seed infection incidence of 0.1-5%, 9.6% showed 5.1-10%, 4.3% showed 10.1-15%, again 4.3% showed 15.1-20%, and 3.2% showed severe seed infection >20%.

Heavily infected seed lots, with percentage infection greater than 15% were recorded from Ada, Ziquala, Minjar and Kulumsa (Table 2) and these heavily infected seed lots comprise 7.4 % of the total seed lots tested. These areas represent the mid-altitude (<2300m) field pea growing agroecology where the temperature is slightly higher than other areas during the growing period. In these places field pea flowers and matures earlier than other places. The lowest infection was found from Ambo, Chelia and Goha Tsiyon with less than

5% seed infection, where these locations are high altitude growing areas. The temperature is lower and the crop matures slowly in these high altitude areas. In all the eleven areas surveyed, at least 30% of the samples were found infected with *A. pinodes*. Thus no production area was found free of this pathogen.

Mean seed infection of the seed lots varied considerably between locations (Table 2). The highest mean seed infection of 8.6% was recorded from Ziquala followed by Minjar with 6.7% where mostly an improved variety (cv. Mohanderfer) was widely grown and these places share similar climatic and cultural conditions because of their proximity. In contrast, low percentage of seed infection was recorded from Ambo (0.7%), Chelia (1.1%) and Goha Tsiyon (1.0%) that are adjacent areas with very high elevation and cool climate. The remaining areas had varying degree of seed infection levels.

There was no specific trend in seed infection between the improved and farmers' cultivars. In areas such as Inewari, Kulumsa and Ziquala, farmers' cultivars had higher seed infection while in the rest areas the improved varieties had higher seed infection (Table 3). Furthermore, very severe seed infection in both improved and farmer' cultivar was found in Ada and Ziquala areas while moderate infection on both group of cultivars was recorded at Chelia and Welmera. Samples from Minjar were mostly improved variety.

Table 2. Incidence of seedborne *Ascochyta pinodes* in 94 field pea seed lots collected from farmers in central Ethiopia in 2002 and tested by blotter method for 12 days under 12 h alternating light and dark at 23+2^{0}C in incubator

Area	Total Samples Tested	Number of samples with % infection of *A. pinodes*						Mean (%)
		0	<5	5.1-10	10.1-15	15.1–20	>20	
Ada	19	9	7	0	0	1	2	4.0
Ambo	10	7	3	0	0	0	0	0.7
Chelia	7	3	4	0	0	0	0	1.1
Debre Tsige	11	4	5	2	0	0	0	1.7
Goha Tsiyon	3	1	2	0	0	0	0	1.0
Inewari	7	3	3	1	0	0	0	2.1
Kulumsa	10	4	3	1	1	1	0	4.9
Minjar	6	2	1	1	1	1	0	6.7
Sendafa	5	2	2	1	0	0	0	2.0
Welmera	8	3	3	0	2	0	0	4.0
Ziquala	8	2	1	3	0	1	1	8.6
Total	94	40	34	9	4	4	3	3.5

Table 3. Mean percentage occurrence of *A. pinodes* in seed lots of farmers' cultivar and improved variety as a result of seed incubation under 12 h alternating light and dark at 23±2^{0}C in incubator for 12 days

Survey area	Farmers' cultivars		Improved variety	
	Range	Mean	Range	Mean
Ada	0 – 23	2.9	3 – 21	5.7
Ambo	0 – 1	0.3	0 – 5	2.5
Chelia	0 – 2	0.6	2 – 3	2.5
Debre Tsige	0 – 6	1.1	-	8.0
Inewari	0 – 7	2.4	1 – 2	1.5
Goha Tsiyon	0 – 2	1.0	-	-
Kulumsa	0 – 16	5.0	-	4.0
Minjar	0 – 0	0.0	0 – 18	8.0
Sendafa	0 – 3	1.0	-	6.0
Welmera	0 – 12	2.7	3 – 13	8.0
Ziquala	0 – 19	9.0	0 – 24	8.3

Comparison between blotter method and seedling symptom test showed that blotter method gave slightly higher incidence of the fungus than seedling symptom test (Table 4). Mean *A. pinodes* incidence was 3.4% on blotter method while 2.6% on seedling symptom test in the greenhouse, which was 23.5% lower than the blotter. In the seedling test, plants were washed and assessed on the basis of symptoms at the seed attachment position of the plant.

Germination percentage of the seed lots was very high in almost all cases where most seed lots had higher than 96% (Table 4). However, seeds from Sendafa area had the lowest mean germination percentage of 92.2% without any spectacular fungi occurrence. All seeds collected from Sendafa, and about half of the seed lots from Ada, Minjar and Ziquala were severely infested with insect pest (pea bruchid) identified as *Bruchus pisorum* (L.). These are adjacent and slightly warmer areas in the surveyed part of the country. There was no correlation between seed infection by *A. pinodes* and germination percentage of field pea seeds.

Table 4. Prevalence of *A. pinodes* and germination percentage of 94-field pea seeds from farmers in central Ethiopia; as a result of seed incubation under 12 h alternating light and darkness at 23±2°C for 12 days and in greenhouse at 20±5°C for 21 days

Survey area	Number of samples	*Ascochyta pinodes* (%)		Germination (%)
		Blotter method	Seedling test	
Ada	19	4.01	2.47	98.2
Ambo	10	0.70	0.40	97.9
Chelia	7	1.14	0.71	97.0
Debre Tsige	11	1.73	1.00	97.8
Goha Tsiyon	3	1.00	1.33	99.3
Inewari	7	2.13	0.86	96.6
Kulumsa	10	4.90	4.70	99.6
Minjar	6	6.67	4.67	96.3
Sendafa	5	2.00	1.60	92.2
Welmera	8	4.00	3.00	98.6
Ziquala	8	8.63	7.50	97.1

There was a strong and positive linear relationship between the blotter method in the laboratory and seedling symptom test in the greenhouse for *A. pinodes* (Figure 2). The seedling infection (dependent variable) was significantly predicted by the blotter method (independent variable) with equation $Y = 0.8429X - 0.2611$ ($df = 92$ and $R^2 = 0.9408^{**}$) where Y was diseased seedling and X was seed infection in the regression model. Either method could be used for assessing this pathogen in field pea seed.

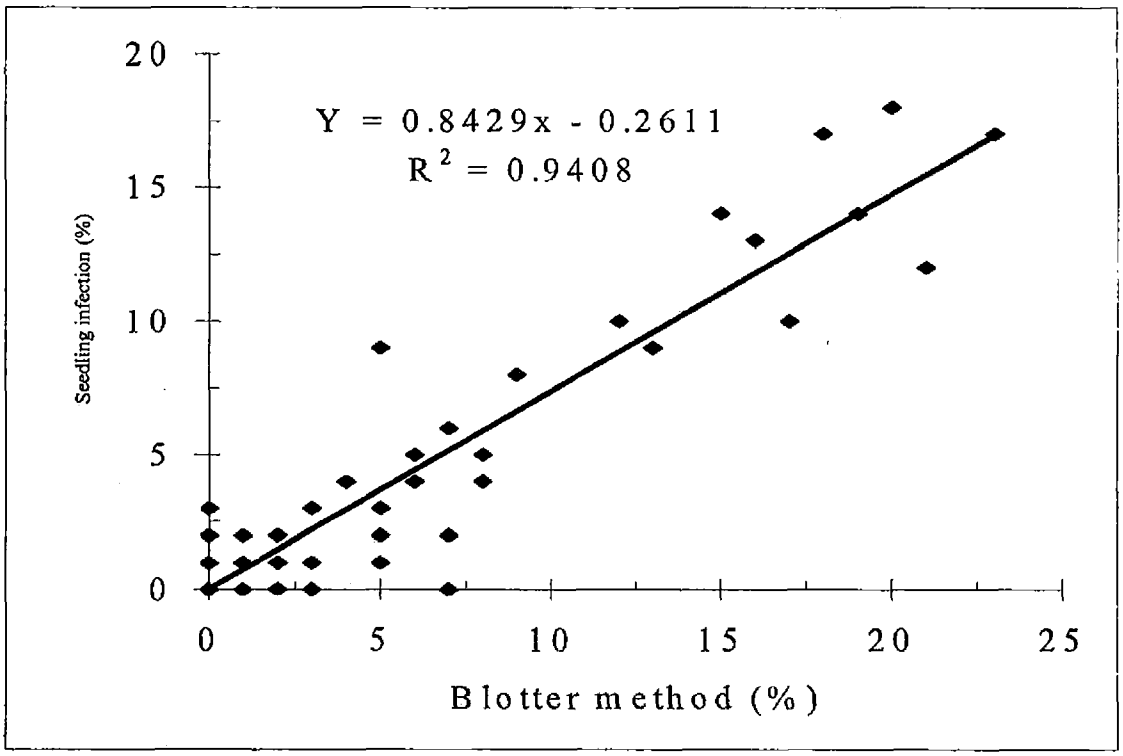

Figure 2. Relationships of seedling infection by *A. pinodes* in greenhouse and seed infection on blotter method in laboratory as a result of assessing 94 farmer seed samples collected from eleven areas in central Ethiopia in 2002.

Discussion

A. pinodes, the major pathogen responsible for Ascochyta blight in field pea was observed to occur at high prevalence, 57.4% of the seed samples being infected at varying degree in central Ethiopia. The incidence in individual seed lot ranged from 0 to 23%. Until this study, little was known concerning the incidence of seedborne *A. pinodes* in the country. Altitude markedly affected the incidence of seedborne inoculum of this pathogen as all the seed lots with >15% incidences were from mid-altitude growing areas (≤2300 m elevation). This high prevalence of *A. pinodes* suggests that this pathogen is widely distributed in central Ethiopia and probably also in other areas. This is a clear indication for its seedborne nature. Infection level above 30% was reported (Bretag *et al.* 1995; Michal *et al.*, 1998; Moussart *et al.*, 1998) but incidence levels from 0 to 5% were most common in seed lots studied. Since seeds from all places harbor this destructive pathogen and 7% of them were severely infected, seeds for planting need to be checked for this fungus to qualify the quality of planting seeds. This emphasizes the need for seed health testing for this pathogen in seed certification, plant quarantine, germplasm management and improved field pea production in the country. Frequent rain favors dissemination and infection of blight disease in field pea and yield was correlated with pod infection (Beasse *et al.*, 1999), which has high correlation with seed infection. Therefore, infection of seed is likewise favored by rainy wet season when the foliage including the pods severely infected. Greatest seed infection occurred when plants were inoculated before and at mid-flowering stages (Xue *et al.*, 1997) and protection of early infection was recommended. Infected seed was among major means by which this pathogen survives and spread to new area or field, and hence, the use of disease-free seed is very important.

survives and spread to new area or field, and hence, the use of disease-free seed is very important.

Another important point that has emerged from the present survey is that field pea seeds looking healthy and well filled are not necessarily free of *A. pinodes* as there was no any symptom associated with this pathogen. However, smaller sized seeds from the same seed lot were mostly free of the pathogen as observed from component platting. Because, they were formed in the upper parts of crop canopy where there was no ascochyta infection and these seeds were formed during drier period of the remaining season. This seems convincing because late time seeds develop and mature during dry period of September and October, the seed size was reduced while early-formed seeds in August were larger in size and the prevailing rainy weather favors blight infection and development. This is the reason why large size seeds of the same seed lot show high incidence of this pathogen and small size ones were free of infection. This doesn't apply to those malformed ones and shriveled due to infection. Seed infection by this pathogen to reduce size was reported (Bretag *et al.*, 1995). Thus seed production programs in these places should consider the flowering period of the crop to reconcile with infection time and seed sorting by size may be important. Gorfu (2004) found that there was a strong and positive correlation between seed infection and pod infection of *A. pinodes* and hence recommended field inspection should consider pod infection rather than total foliage infection by *A. pinodes*. Chen *et al.* (1994) studied on the mechanism of seed infection and showed that the fungus appeared to penetrate into seeds directly through pods and young pods being more susceptible. However, critical analysis in the influence of environmental factors upon disease progress and infection of seed is lacking.

Germination is important indicator of seed quality and in this study germination of seeds was very high for all except those collected from Sendafa area. These samples were exceptionally damaged by *Bruchus pisoroum* (L.) thus the germination percentage was very low. The pest was confounded to only Ada, Minjar and Sendafa, which are adjacent places. There was no earlier report on the occurrence of pea bruchid for the survey area. No decrease of germination was observed due to *A. pinodes* in our study while poor germination was reported due to this pathogen elsewhere (Bretag *et al.*, 1995; Xue *et al.*, 1996).

No seed sample was found treated with any kind of protection mechanism or product indicating the low development stage of seed maintenance and quality control in the area. Seeds were handled with grains in all cases.

Finally, the wide spread and varying degree of seed infection by *A. pinodes* would probably indicate the presence of different level of resistance in the local materials. Since Ethiopia is the secondary center of diversity for this crop and farmers grow landraces for centuries, resistance for seedborne inoculum is expected in these local materials. Further studies are required to clarify this aspect of Ascochyta blight.

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