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Spatial distribution of *Aphanomyces euteiches* inoculum in a naturally infested pea field

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Abstract The main objective of the study was to describe the horizontal and vertical distribution of *Aphanomyces* root rot in a naturally infested pea field. Measurements of inoculum potential clearly indicated a horizontal distribution of inoculum among several foci in the field, these foci differing in size and disease intensity. A highly significant relationship was observed between disease severity on plants during the cropping season and soil inoculum potential. In terms of the vertical distribution of inoculum in the soil, detection was maximal at a depth of 10 to 40 cm, but inoculum was detected down to a depth of 60 cm. Generally, inoculum potential was lowest for the layers at depths of 0 to 10 and 50 to 60 cm. Inoculum distribution and the value of the methodologies used are discussed in terms of possible use for epidemiology and disease forecasting.

Keywords Soilborne disease · Inoculum potential · Disease severity · Horizontal and vertical distribution

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Introduction

Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches* is a major root disease of pea (*Pisum sativum*) that has been reported in many pea-growing areas of the world (Kraft and Pfleger 2001; Levenfors 2003). This soil-borne pathogen induces severe rotting of the root system, often resulting in typical yellowing and stunting of the aerial parts of the plant. The losses caused by *A.*

euteiches are variable, depending on temperature and moisture conditions; entire fields may be destroyed during wet and warm years (Papavizas and Ayers 1974). In France, this disease has caused heavy losses since 1993 (Wicker et al. 2001) and led to a decrease in the use of pea crop acreage. Long rotations and the avoidance of infested fields are the only practical measures currently available to control this disease. *Aphanomyces euteiches* oospores are reported to survive in soil and to remain virulent for 10 to 20 years in the absence of susceptible crops (Papavizas and Ayers 1974; Pfender and Hagedorn 1983). They can also resist adverse conditions, such as alternate freezing and thawing in dry conditions (Sherwood and Hagedorn 1962). Consequently, long rotations may be required to reduce *A. euteiches* infestation to safe levels. Rotations of 5 to 15 years may be required, depending on several factors, including initial level of infestation, soil type and climatic conditions (Papavizas and Ayers 1974; Sundheim and Wiggen 1972).

Inoculum potential (IP) was defined by Malvick et al. (1994) as an index of potential disease activity combining propagule infectivity, propagule density, and soil factors inhibiting or promoting root infection. Sherwood and Hagedorn (1958) and Chan and Close (1987) developed a baiting technique to evaluate the IP of soils naturally infested with *A. euteiches*. Hagedorn (1984) found a strong correlation between results obtained in this test and the severity of root rot developing in the field. However, despite the existence of techniques of this kind, very little is known about the spatial distribution of *A. euteiches*.

Diseased plants in infested fields are often seen to form foci of various sizes. According to Burke et al. (1970), this organism predominates in the layer of ploughed soil. However, Kraft et al. (1990) found it up to 60 cm down in the soil profile. Consequently, the main objective of this study was to investigate the precise horizontal and vertical distribution of *Aphanomyces* root rot, based on disease severity (DS) data. Horizontal distribution was assessed by using two different methods for mapping in a single field: a baiting technique to determine the IP of the soil and an evaluation of DS on pea plants during the cropping period. Moreover, we investigated the vertical distribution of the fungus by determining the IP of different soil layers in several plots of the studied field.

Materials and methods

Site description

The study was conducted in a field at the *Union Nationale Interprofessionelle des Legumes Transformés* (UNILET) farm, in Brittany (Riec sur Belon, Finistère), France, reported to be naturally infested with *A. euteiches* (Le Delliou, personal communication). This field, with a well drained sandy silt soil, had not been planted with peas for at least 10 years and had a cropping history of wheat and maize since 1990. The field (0.92 ha) was divided into contiguous quadrats of 10 by 10 m. Grids were marked with plastic stakes so that the precise location of each quadrat could be identified after each cropping intervention.

Horizontal distribution of *Aphanomyces* root rot

Inoculum potential of the soil before the beginning of the cropping season

There is no direct method for quantifying *A. euteiches* propagule density in soil. We therefore carried out a bioassay to measure IP. This bioassay was adapted from the soil indexing method developed by Sherwood and Hagedorn (1958). Soil samples were collected in November 2000 before the beginning of the spring cropping season in order to determine the horizontal inoculum distribution. Five soil subsamples were removed at equal distances apart from each other (2.5 m) from the left diagonal of each quadrat, with a spade (subsamples 5 cm thick and 20 cm deep). The tools used for soil collection were cleaned between quadrats. The soil subsamples from each quadrat were combined in a plastic bag labelled with the coordinates of the quadrat. Soil samples were broken up manually and stones and other debris were removed, to ensure that the soil was thoroughly mixed. Each well mixed soil sample was used to fill four plastic pots (500 ml), and five pea seeds of cv. Baccara (Ets Florimond Desprez, France), susceptible to *A. euteiches*, were sown in each pot. Pots were arranged in a completely randomised design and maintained in a climatic chamber (thermoperiod=25/23°C and photoperiod =16/8 h, with a light intensity of 160±2 μE m^{-2} s^{-1}). The soil was kept moist by regularly watering to favour disease. Fourteen days after sowing, plants were removed and their roots were carefully

washed under running tap water. IP was assessed, on a scale from 0 to 5 (Wicker et al. 2001): (0) no symptoms; (1) discoloured traces on rootlets; (2) discoloured to honey-brown zones covering at least half of the root system; (3) honey-brown, soft zones covering at least half of the root system; (4) most of the root system soft and honey-brown to dark brown; (5) plant dead. IP was assessed for each quadrat, making it possible to visualise the spatial distribution of the disease, and a map was generated for the studied field.

Disease severity during the cropping period

Peas (cv. Baccara) were sown (80 seeds m^{-2}) on 19 April 2001. Disease severity was assessed about 1 month after detection of the first root rot symptoms (12 June 2001). At this time, no symptoms due to other root-invading pathogens, such as *Fusarium* spp., were present. We removed 20 plants from the left diagonal of each quadrat. Roots were carefully washed and examined for typical symptoms on primary and lateral roots. Infected areas were softened, watersoaked and slightly discoloured. DS was scored on the scale of 0 to 5, previously described for IP. As described above, a map was generated. The relationship between the results of IP analysis and DS in the field was evaluated, using Pearson's correlation analysis (SAS 1989).

Vertical distribution of the inoculum

The vertical distribution of inoculum was investigated in quadrats corresponding to different levels of IP ($0<IP\leq1$, $1<IP\leq2$, $2<IP\leq3$ and $3<IP\leq4$). Three quadrats were arbitrarily chosen for each level of IP:

$$
\begin{aligned}
0 < \mathrm{IP} \leq 1 &: \text{quadrats } 76(\mathrm{IP} = 0.61),\\
&\quad 49(\mathrm{IP} = 0.75) \text{ and } 92(\mathrm{IP} = 0.55).\\
&\quad \text{Mean IP} = 0.64 +/- 0.10\\
1 < \mathrm{IP} \leq 2 &: \text{quadrats } 7\ (\mathrm{IP} = 1.46),\\
&\quad 21(\mathrm{IP} = 1.73) \text{ and } 55(\mathrm{IP} = 1.38).\\
&\quad \text{Mean IP} = 0.62 +/- 0.18\\
2 < \mathrm{IP} \leq 3 &: \text{quadrats } 2\ (\mathrm{IP} = 2.50),\\
&\quad 33(\mathrm{IP} = 2.85) \text{ and } 40(\mathrm{IP} = 2.71).\\
&\quad \text{Mean IP} = 2.69 +/- 0.18\\
3 < \mathrm{IP} \leq 4 &: \text{quadrats } 34\ (\mathrm{IP} = 3.24),\\
&\quad 62(\mathrm{IP} = 3,74) \text{ and } 74(\mathrm{IP} = 3.22).\\
&\quad \text{Mean IP} = 3.40 +/- 0.29.
\end{aligned}
$$

Soil samples were taken on 5 March 2001, at depths of 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50 and 50 to 60 cm, using a drill. For each quadrat, five subsamples were collected for each depth and pooled in a plastic bag labelled with the quadrat and depth co-ordinates. The tools used for soil collection were cleaned between layers and between quadrats. The bioassay described above was used to assess the IP of soil samples from each depth in each quadrat in the laboratory.

For each quadrat, mean IP was calculated for each depth and an analysis of variance (ANOVA) was carried out in order to compare IPs between depths. Means were compared, using a Newman–Keuls test ($P=0.05$) in the General Linear Model procedure of SAS (1989) (SAS Institute, Cary, NC, USA).

Results

Horizontal distribution of *Aphanomyces* root rot

Inoculum potential

Symptoms on pea seedlings in the bioassay were typical of *A. euteiches* and the pathogen was readily isolated from infected tissues. The spatial pattern of IP in the 92 quadrats is illustrated in Fig. 1. *Aphanomyces euteiches* was not detected in quadrat 9. In the other quadrats, IP was low to very high, depending on the area of the field. Thirty-six quadrats had an IP of 0 to 1, 33 had an IP of 1 to 2, 8 had an IP of 2 to 3 and 14 had an IP of 3 to 4. Two main foci with a high IP ($IP>3$) were observed: the first covered an area of 800 m^2 (40×20) and the second covered an area of 1600 m^2 (20×80).

Disease severity

The symptoms observed on sampled plants were typical of *A. euteiches*. All the sampled plants were diseased, but DS varied between quadrats (Fig. 1). Four quadrats had a DS of 0 to 1, 64 had a DS of 1 to 2, 10 had a DS of 2 to 3 and 14 had a DS of 3 to 4. These last 14 quadrats corresponded to the two parts of the field with the highest IP.

When IP values exceeded 1.5, a significantly positive correlation ($r^2=0.86$; $P<0.01$) was observed between IP values and DS in the field (Fig. 2). For IP

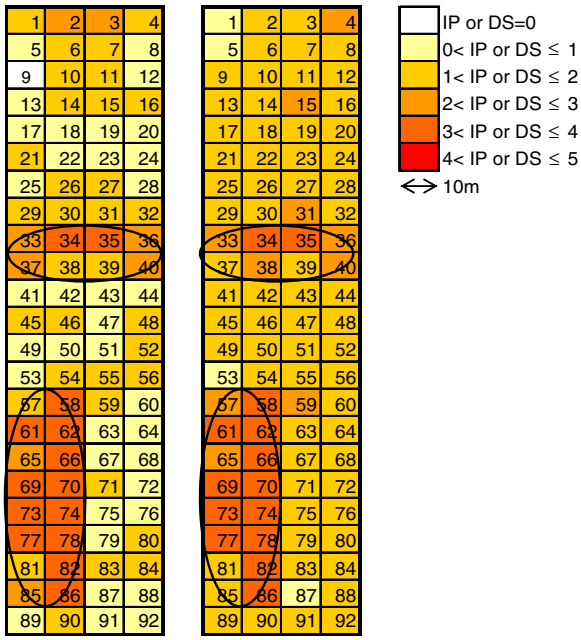

Fig. 1 Mapping of the field, using inoculum potential values (*IP*; *left*) and disease severity values (*DS*; *right*). Main focuses are *circled*

values below 1.5, mean disease severity in the field was between 1 and 2 and no correlation was observed.

Vertical distribution

Inoculum was always present down to a depth of 60 cm, regardless of the IP of the quadrat concerned (Fig. 3). For the four classes of IP studied, the uppermost (0–10 cm) and lowermost (50–60 cm) layers had the lowest IP. Mean IP was greatest at 10–30 cm, decreasing with increasing depth thereafter. The higher the IP was, the higher it was in depth.

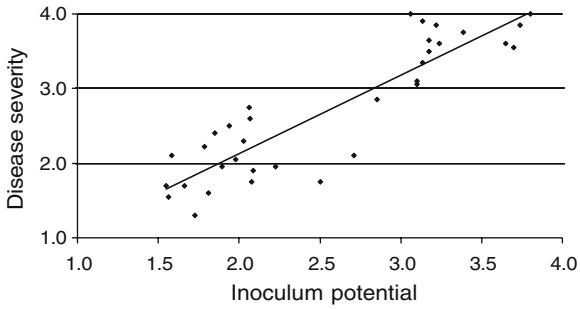

Fig. 2 Relationship between disease severity in the field and inoculum potential

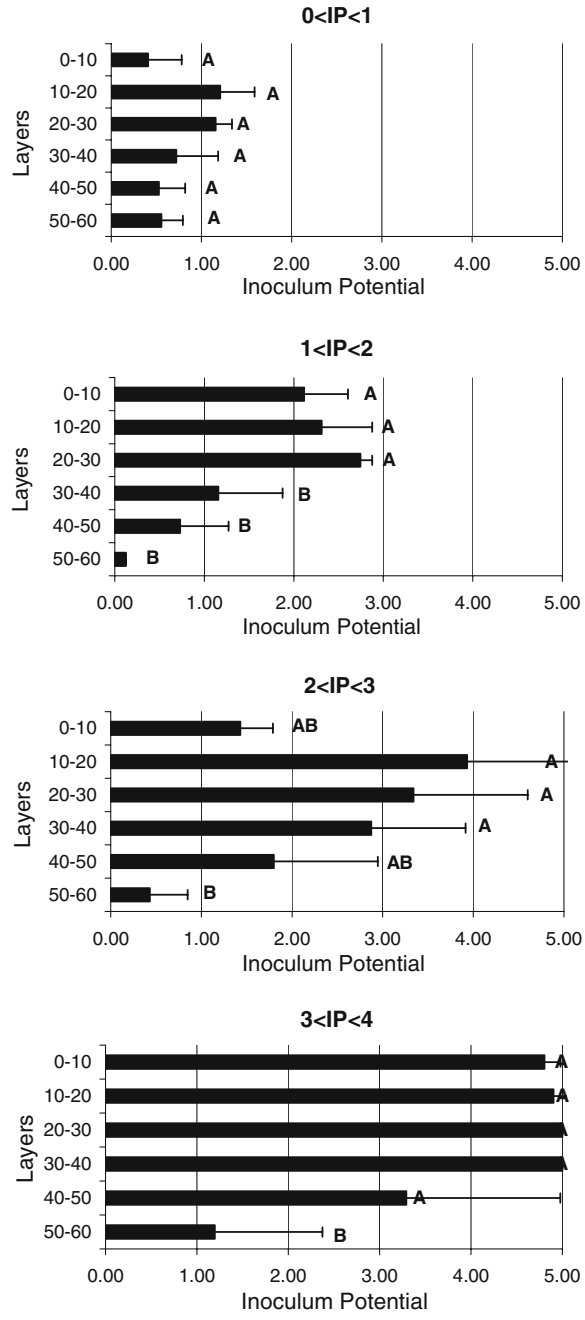

Fig. 3 Vertical inoculum distribution for four levels of inoculum potential (*IP*). Three quadrats were studied for each level of IP. Mean values followed by the same letter are not significantly different (Newman–Keuls test $P=0.05$)

Discussion

The results obtained in this study provide information about the spatial distribution of *A. euteiches* inoculum in the soil of infested fields. To our knowledge, it is

the first time that horizontal and vertical inoculum distributions have been precisely described in a field.

IP mapping clearly showed that inoculum was distributed in several foci, of different sizes and disease intensities, within the plots. The main focus observed was localised in the lower part of the field where soil moisture retention was high. This result is in accordance with the observations made by Pfender and Hagedorn (1983) and Kraft et al. (1990). Many previous studies have reported the marked aggregation of soilborne pathogens in soil. For example, similar distributions have been observed for the wheat/*Gaeumannomyces graminis* var. *tritici* (Gosme et al. 2006) and pepper/*Phytophthora capsici* (Larkin et al. 1995) pathosystems. The field studied here had not been sown with peas for at least 10 years. Our results therefore confirm that inoculum may persist for very long periods of time in the soil, as previously reported. Sherwood and Hagedorn (1962) showed that rotations of 5 or 6 years were often insufficient to reduce *A. euteiches* infestation to safe levels, due to the persistence of oospores in the soil, facilitating pathogen survival.

There was a highly significant relationship between DS on plants during the cropping period and IP (bioassay). Mapping of disease assessment scores on the pea crop could be used to study the distribution of inoculum in a field. This method is less tedious than IP mapping, but it may be also less precise, because it depends on climatic conditions and the period of disease assessment.

On all plots other than those with the highest IP, the 0–10 cm layer of the soil generally had a low IP. This may reflect low levels of inoculum maintenance due to hot dry periods, which may affect superficial layers of the soil more strongly than deeper layers, and/or the direct effect of UV irradiation on inoculum. All plots had an IP significantly lower than the mean for soil depths of 50 to 60 cm. This may be due to low levels of stimulation by root exudates at this depth, as a result of the low level of soil colonisation by roots, or the low oxygen content of the soil at this depth. Inoculum levels seemed to be maximal at soil depths between 10 and 30 cm, corresponding to the zone of maximal root exploration and optimal soil conditions for inoculum storage. Voisin et al. (2002) have shown that rooting depth—corresponding to the depth above which more than 70% of the counted roots were located—does not generally exceed 30 cm. These results were consistent with those obtained by Pfender and Hagedorn (1983) who observed that *A. euteiches* inoculum, generally associated with organic debris, was found primarily in the layer of ploughed soil, in which it may persist for >10 years. Our observations on the vertical distribution of *A. euteiches* in the soil, were also similar to those made for two plant-parasitic nematodes: *Heterodera glycines* (Rupe et al. 1999) and *Meloidogyne chitwoodi* (Wesemael and Moens 2007).

This research should be extended for several reasons. We studied IP at soil depths down to 60 cm, but the pathogen could presumably be found at lower levels. Moreover, this study was carried out in a well drained sandy-silt soil. IP at different depths may depend on soil characteristics and should therefore be studied in different types of soil. No method is currently available for quantifying inoculum density. We used an indirect method, but more direct methods, such as the qPCR method developed by Sauvage et al. (2007) may be more precise.

Effective control measures are required for the cultivation of peas in areas infested with *A. euteiches*. This method for determining IP based on the use of baiting plants efficiently predicts the infectivity of the soil in a given field. French pea growers currently use this method to identify fields suitable for pea crops before sowing (Moussart et al. 2006). Characterisation of the spatial distribution of the disease at field level is particularly valuable for the formulation and evaluation of control strategies. The development of effective sampling methods is essential (Mihail and Alcorn 1987), as this may make it possible to study the influence of environmental and cultural factors on population dynamics (Olanya and Lee Campbell 1988; Royse et al. 1999), or to determine the relationship between IP, DS and yield losses (Hughes 1990).

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