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Aphanomyces euteiches as a component of the complex of foot and root pathogens of peas in Dutch soils

PEDRO OYARZUN[1]* and JOOP VAN LOON[2]**

[1] Research Station for Arable Farming and Field Production of Vegetables (PAGV), P.O. Box 430, 8200 AK Lelystad, the Netherlands
[2] Foundation for Agricultural Plant Breeding (SVP), P.O. Box 117, 6700 AC Wageningen, the Netherlands

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Abstract

The occurrence of *Aphanomyces euteiches* Drechs. in Dutch soils is reported for the first time. Isolates of the pathogen were obtained from peas (*Pisum sativum* L.). A bioassay was used that baited the pathogen from soil into the cortex of stem and root of seedlings of a highly susceptible pea cultivar. The pathogen could subsequently be isolated on a semi-selective medium. Screening of soil samples from 13 fields known to be infested with fungi causing foot and root rot demonstrated the presence of *A. euteiches* in 10 cases. In a second screening on soil samples from 43 fields, the pathogen was present in 16 cases. A positive correlation was found between the disease severity caused by *A. euteiches* in the seedling bioassay and the disease severity caused by the complex of foot and root pathogens in the same soils as evidenced by a mature plant bioassay. It is considered probable that *A. euteiches* has since long been a common component of the foot and root rot complex in Dutch soils but has not been detected previously due to inadequate sampling and isolation techniques.

Additional keywords: Pisum sativum L., bioassay.

Aphanomyces euteiches Drechs. is considered a specialized pathogen of Leguminosae. It is one of the most destructive pathogens in the complex of foot and root rot pathogens of peas, *Pisum sativum* L. (Papavizas and Ayers, 1974; Pfender, 1984). It causes a soft rot of the entire root system that is known in the USA as 'common root rot' of peas. The economic loss caused by common root rot can be severe. In the United States, for green peas average yield losses in fields infested with common root rot have been estimated at 25-30% (Papavizas and Ayers, 1974).

Due to its economic importance *A. euteiches* is one of the best studied fungal diseases of the pea. The literature on its taxonomy and ecology has been extensively reviewed by Papavizas and Ayers (1974).

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* Present address: Research Institute for Plant Protection (IPO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands.
** Present address: Department of Entomology, Wageningen Agricultural University, P.O. Box 8031, 6700 EH Wageningen, the Netherlands.

A. euteiches is a cosmopolite and it has been reported from several European countries including Denmark (Gram et al., 1929), France (Labrousse, 1934), Great Britain (Beaumont, 1951), Sweden (Olofsson, 1967) and Norway (Sundheim and Wiggen, 1972). In the etiology of foot and root rot of peas in the Netherlands, *A. euteiches* has not been mentioned so far (Buisman, 1927; Riepma, 1952; Labruyère and Van der Spek, 1962).

In the course of a research focussed on a bioassay to predict the risk for foot rot in dry-harvested peas, the symptoms described by Papavizas and Ayers (1974) were repeatedly observed in test plants of cv. Finale (Oyarzun, 1989). Using routine media like potato dextrose agar and water agar, mainly *Pythium* spp. were isolated from these plants. In June 1988, a set of pea varieties was screened for their suitability as test plants for the bioassay. The cultivar Marzia displayed a clear softrot. When sections from the stem base were exposed to tap water, zoosporangia developed and zoospores extruded within 36 hours. Inside the cortical tissue oospores with a morphology reminiscent of those of *A. euteiches* were observed.

The availability of a susceptible commercial variety, a bioassay that is described hereafter (J.M. Kraft, pers. comm.) and the experience with a suitable isolation technique, led us to reexamine soil samples from grower's fields that had previously been assayed for their foot rot potential in a mature plant bioassay. Thus, we aimed to establish as to how far *A. euteiches* was involved in the complex of foot and root pathogens.

Two series of soil samples were investigated for their infestation with *A. euteiches* in two separate bioassays. The first series consisted of 13 samples that had been found to produce soft rot in mature plant bioassays. Ten of these had been collected directly from grower's fields in March 1987, two in March 1986 and one was taken from an experimental field in the Eastern Flevoland Polder. On the latter field, only peas had been grown continuously from 1979 to 1988. On this soil, the characteristic symptoms of soft rot had developed in cv. Marzia in the varietal test. Samples had been collected by pooling 50 subsamples of 0.3 litre taken from the upper 20 cm soil layer at random from a one hectare surface of each field. The pooled samples were crumbled, thoroughly homogenized and sieved (8 mm). They were then stored in plastic bags at 5 °C in the dark. The bioassay was performed in September 1988.

The second series consisted of 43 samples taken from grower's fields in December 1988. Collection, processing and storage were identical to what has been described for the first series. These were bioassayed for their infestation with *A. euteiches* in February 1989. A bioassay with baiting plants for *A. euteiches* was employed that provides an indication on inoculum potential. Seed of peas cv. Marzia was superficially disinfested at room temperature by a 15 min soak in a 1% hypochlorite solution and subsequent washings in distilled water. It was pregerminated in petri-dishes lined with moist filter paper during 5 days at 20 °C. Healthy seedlings with tap roots of 4 cm length were placed on two layers of moistened filterpaper in petri-dishes of 15 cm diameter, three seedlings per dish. The seedlings were covered with soil (ca. 15 ml per petri dish). The dishes were incubated at 26 °C and 100% rh in the dark during 7 days. After this period, the seedlings were assessed for disease symptoms and tissue was excised to isolate fungi from it. Isolation and purification was performed on a semi-selective dilute corn agar medium (Pfender, 1984).

Soft rot symptoms developed in the seedlings within 7 days in 10 out of the 13 samples (77%) assayed in the first bioassay and in 16 out of 43 samples (37%) in the second

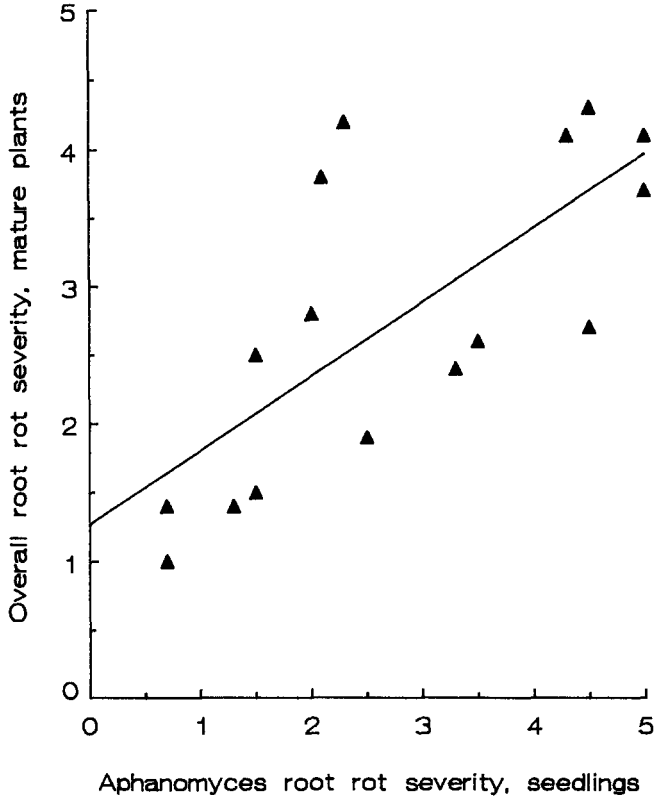

Fig. 1. Disease index assigned in the second bioassay on the inoculum potential of *A. euteiches* root rot in 16 soil samples (mean of three plants per sample; test plants were seedlings of pea cultivar Marzia) plotted against the disease index of overall root rot in a mature plant bioassay (mean of 30 plants of the cultivar Finale) in the greenhouse. Index scale from 0 (root system and epicotyl healthy) to 5 (root and epicotyl severely rotted, leading to death of plant). Regression line: $y = 0.58x + 1.31$.

one (Fig. 1). In the first bioassay, in 7 of the 10 cases, severe rotting of the cortex causing a translucent yellow-goldish discoloration of the tissue was observed. This rotting caused the death of the seedlings soon afterwards. This included both samples collected in 1986 and the one from the field with continuous pea cropping. In the other three cases, symptoms of soft rot were moderate but clearly visible. For comparison, the mean foot rot indices found for the same soils in a previous mature plant bioassay (Oyarzun, 1989), carried out in January 1989, are given. The latter bioassay involved the testing of soil from grower's fields, that had been collected, prepared and stored in a way identical to the description given earlier. The soil was then potted in 3 litre containers and such an amount of water was added as to reach field capacity, which had been determined previously for each soil. Moisture content of the soil was kept at field capacity by regular weighing of the pots and addition of the necessary amount of water. Twelve seeds of cv. Finale, taken from a lot tested to have less than 1% diseased seeds, were sown in each container. Each soil was represented by four replicate containers. The

bioassay was performed in a greenhouse at 18-24 °C under natural lighting. At the onset of flowering, 4-5 weeks after sowing, the soil was carefully washed from the roots of the test plants and the severity of foot and root rot disease was assessed visually. Severity was expressed in a disease index which ranged from 0 where no symptoms of foot and root rot were observed to 5 where the root system showed a severe rot leading to the death of the plant. For the seven samples inducing severe rotting, the mean mature plant disease index was 3.4, for the three samples showing moderate rotting 2.3 and for the three remaining samples apparently free from *A. euteiches* this was 2.2. Thus, it seems that the indicated level of *A. euteiches* is positively correlated with the higher foot rot indices found previously for these soils in the mature plant bioassay.

Previous large scale isolations from diseased plants from this bioassay consistently gave abundant numbers of four pathogenic fungal species traditionally known to be causal agents of foot and root rot in peas (Riepma, 1952; Oyarzun, 1989) but till now in no case *A. euteiches* had been identified.

In all 10 cases that showed soft rot symptoms on a macroscopical level, large numbers of oogonia and oospores were found in the cortex of tap root and epicotyl upon microscopical examination. Oospores measured 20-25 μm in diameter. Isolations on corn meal agar consistently gave highly similar, sparsely growing arachnoid colonies that abundantly produced oospores upon ageing. The identity of four of these colonies was confirmed as *Aphanomyces euteiches* Drechsler by the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands.

Results of the second bioassay were essentially similar. Disease severity was assessed in more detail than in the first one, using the same scale as that applied in the mature plant bioassay on the corresponding samples. Fig. 1 shows that there is a positive relationship between both indices (Spearman's rank correlation coefficient $\varrho = 0.72$, $P < 0.01$ ($n = 16$)). This suggests that the disease severity found in the mature plant bioassay is in part dependent upon the severity of soft rot in seedlings caused by *A. euteiches*.

In the present paper we report for the first time on the presence of *A. euteiches* Drechs. in Dutch soils. The bioassay used may easily be adapted to establish differences in inoculum potential. The assay was rapid and simple to perform. In this study, a limited number of samples was assayed. The evidence supplied for the possible importance of *A. euteiches* as a root pathogen in peas needs substantiation and deserves extended research. In preliminary inoculation experiments, the purified pathogen showed a high degree of virulence (M. Gerlagh, pers. comm.). In 1987, 27.5% of the 51 samples screened induced soft rot. In 70% of these cases, *A. euteiches* was shown to be present, after 18 months of storage.

The failure of detecting *A. euteiches* in previous mycological research on species associated with foot and root rot of peas in the Netherlands may be explained by the use of isolation procedures that apparently were inadequate for this organism. The failure in isolating *A. euteiches* on commonly used agar media have been documented (Papavizas and Ayers, 1974; Pfender et al., 1984). One factor that may have played a role is the use of mature plants, i.e. in the flowering or pod stage, to obtain pathogen isolates. In the mature plant phase, the invasion of several species of secondary pathogens evidently interferes with the demonstration of *A. euteiches*.

Although the detection of *A. euteiches* reported here contributes to insight into the causes of the present incidence of foot and root rot of peas in Dutch soils, we feel that

it is not a new threat to the pea crop. In previous Dutch research on foot rot in peas, soft rot was repeatedly noticed where unidentified phycomycetous species were involved (Riepma, 1952; Labruyère and Van der Spek, 1962). It is well possible that *A. euteiches* has been present already from the start of large scale pea growing on, as in other parts of Europe and North America.

There are no effective curative control measures against *A. euteiches*. Cultural practice should aim at prevention of inoculum build-up to damaging levels, by observing an adequate interval between two pea or, which may be even more safe, two legume crops. An additional strategy to prevent yield loss caused by *A. euteiches* is the growing of pea varieties resistant to this pathogen (Shehata et al., 1983; Kraft, 1988; Lewis and Gritton, 1988). Breeding pea varieties for dry harvest with resistance to foot and root rot (Van Loon et al., 1988) should pay due attention to *A. euteiches*.

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Samenvatting

Aphanomyces euteiches: een component in het voetziekte-complex van erwten in Nederlandse gronden

De aanwezigheid van *Aphanomyces euteiches* Drechs. in Nederlandse gronden is voor het eerst aangetoond. Isolaten van het pathogeen werden verkregen van erwten (*Pisum sativum* L.). De pathogene schimmel werd in petrischalen uit grond in het schorsweefsel van wortel en stengel van een zeer vatbaar erwteras gelokt. Met behulp van een semiselectief medium konden vervolgens isolaten van de schimmel worden verkregen. Toetsing van grondmonsters afkomstig van 13 percelen, waarvan bekend was dat ze besmet waren met schimmels die voetziekten in erwten veroorzaken, toonde de aanwezigheid van *A. euteiches* aan in 10 gevallen. In een tweede biotoets op grondmonsters van 43 percelen bleken 16 monsters het pathogeen te herbergen. Er werd een positieve correlatie gevonden tussen de ernst van de aantasting door *A. euteiches* van kiemplanten en de aantasting van volwassen planten in een biotoets in de kas. Het is waarschijnlijk dat de schimmel reeds lang in Nederlandse akkers voorkomt, maar door inadequate bemonsterings- en isolatietechnieken over het hoofd is gezien.

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