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Leaf and head rot of Chinese cabbage – a new field disease caused by *Pythium tracheiphilum* Matta

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Abstract

A new disease has arisen in field grown Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) in Denmark. The soil-borne causal agent was shown to be *Pythium tracheiphilum* Matta, a little known pathogen of lettuce. Until now, outbreaks of the disease in Chinese cabbage were generally attributed to *Phytophthora porri* Foister, since symptoms of the disease in the field resemble those caused by *Ph. porri* in Chinese cabbage in cold stores. In some severely affected crops harvest losses in 1994, directly attributable to *P. tracheiphilum*, were estimated to about 50%, corresponding to a market value of up to $ US 20000 per ha. Outbreaks of the disease coincide with the heading of the crop, while juvenile plants or seedlings are apparently not attacked. It appears that the outer leaves are infected directly from the soil. Mainly above ground parts are affected and colonized by the pathogen, and indications of infection via the root system have not been observed. This is the first record of *P. tracheiphilum* as a pathogen of Chinese cabbage.

Introduction

A new disease problem has arisen in field crops of Chinese cabbage in Denmark. The disease is initially seen as a dry, greyish collapse of tissue in the basal mid-rib area of the outermost leaves forming irregular oval lesions. The lesions spread up the mid-rib into the lamina forming what rapidly becomes a brownish, wet rot as saprophytic bacteria colonise the infected tissue. Due to tissue collapse, leaves drop and dehisce, and the rot progressively enters the leaf whorl, eventually reaching the heart leaves. The onset of the disease closely coincides with the start of head formation. Attack of seedlings or of juvenile plants has never been observed, and plant emergence appears unaffected by the presence of the pathogen in the soil. In Denmark, Chinese cabbage is planted or direct-sown from early May until late June and although disease outbreaks occur throughout the growing season, attacks become more conspicuous later in the season, due to a more rapid decay of affected leaves. Harvest losses of 40 to 50% have been commonly observed, corresponding to loss of up to US $ 20.000 per ha. A view of a field of Chinese cabbage at an advanced stage of attack, showing whitish heads due to the loss of the dark green cover leaves, is shown in Figure 1.

Usually the disease is uniformly distributed in the field rather than radiating from foci, and completely healthy plants juxtapose with severely diseased ones. However, a pronounced field variation may be observed, with the more severe attacks localized in the areas with frequent standing water.

Materials and methods

The disease and its causal agent have been studied as outlined below. Tissue from lesions of diseased plant material from the field was initially inspected by microscopy. For isolation and identification of the causal agent tissue sections of about 0.25 cm^2 were taken from leaf lesion margins and from necrotic vascular tissue of leaves. To examine the extent of colonization, sections were also taken from necrotic

Figure 1. Advanced stage of attack by *P. tracheiphilum* in a field crop of Chinese cabbage.

vascular tissue from leaf traces, stems and upper root (necrotic tissue was not found any deeper) of diseased plants. Tissue sections were incubated at 22–23 °C on plain water agar (1.5%) or on P_5ARP (Jeffers and Martin, 1986). After 2–5 days of incubation emerging hyphal tips were transferred to 1.5% CMA amended with 0.5 ‰ of wheat germ oil (CMAs). Morphological study and species identification was made by microscopy at 20–100 × magnification of 4–10 days old pure cultures on CMAs.

An attempt was made to isolate the disease causing agent from the surface of Chinese cabbage roots, obtained from a Chinese cabbage crop growing in a field in which attacks of the disease have occurred regularly and massively. Due to water stress the crop in question was not yet at the head formation stage, though about 40 days old, and thus no disease symptoms were observed. Tap roots of 100 plants were rinsed carefully under running tap water, then left to air-dry. Roots were then plated separately on P_5ARP and covered by a thin film of plain water agar to ensure overall surface contact to the substrate. After 2–3 days at 20 °C isolates of prevalent mycoflora were obtained by transfer of hyphal tips to CMAs. On this substrate, after at least 4 to 8 days of incubation, all *Pythium* spp. like isolates found were subjected to microscope studies.

Pathogenicity of isolates obtained from typical symptoms was tested by transfer of 1 cm^2 blocks of 2–4 days old cultures on CMAs to the mid-rib of whole, detached, healthy head leaves of Chinese cabbage obtained on the market, i.e. leaves of a physiological age corresponding to that of pre-harvest head leaves. Mid-ribs were surface disinfected prior to inoculation, using 70% ethanol on a cotton swab. Inoculations were made to both non-wounded and to artificially wounded leaves, using a 1 cm^2 block of 2–4 days old culture on CMAs for inoculum. Wounding was made by lifting an epidermal strip with a sterile scalpel. The controls, both wounded and non-wounded leaves, were inoculated with sterile agar blocks. Inoculated leaves were incubated for 7 days at 22–23 °C on moist blotter paper in inflated polyethylene bags and observed daily for symptoms. Re-isolations from developing symptoms were made by transfer of tissue from margins of developing leaf spots to P_5ARP, and verification of identity of the re-isolated organisms was made by microscopy of cultures on CMAs.

Pathogenicity to lettuce was tested using the above method, and pathogenicity to cucumber was also tested

by moist incubation of 5 cm fruit segments inoculated as above, on either the cut side or on the undamaged epidermis.

Symptoms and disease development were followed in several Chinese cabbage fields in southern Zealand during 1993–95. Internal disease progress was observed by inspection of longitudinal and transversal sections of adult plants and roots.

The causal agent

The causal organism of the disease has been found to be the oomycete *P. tracheiphilum*. Microscopy of leaf lesion tissue revealed non-septated hyphae and numerous oospores and sporangia. The pathogen was readily obtained from leaf lesion tissue or from necrotic leaf trace tissues and their (necrotic) vascular extensions into the upper root, as far as 3–5 cm below the first node, when such material was incubated on either P_5ARP or on plain water agar. Isolates corresponded closely to Matta's (1965) description, and their identity has been confirmed by the International Mycological Institute (two isolates have been deposited at IMI, nos. 360389 and 360390).

Isolates of *P. tracheiphilum* obtained from diseased plants in the field reproduced symptoms in pathogenicity tests on detached leaves of Chinese cabbage. Within 3 to 7 days typical 5 to 15 cm long, elliptical lesions had formed. The postulates of Koch were fulfilled since *P. tracheiphilum* was re-isolated from margins of symptoms induced. Symptoms developed on both wounded and non-wounded material, but more consistently so on wounded material. In tests of pathogenicity to both wounded and non-wounded leaves of lettuce water-soaked lesions with collapsed, later brownish tissue and necrotic veins spread from the inoculation site to leaf margins within 3 to 7 days. Cucumber fruit segments inoculated on the cut surface were rapidly and completely colonized and decayed while segments inoculated at the epidermal side displayed no symptoms.

Symptoms and disease development

Initial lesions may be found on mid-ribs a few centimetres distant from the leaf base, indicating soil to leaf infection, but may also originate from the leaf base itself. In the latter case initial lesions are characteristically finger-nail-shaped. In some cases the pathogen evidently passes directly from a lesion on one leaf through the epidermis of an adjacent, inner leaf, but generally the pathogen seems to spread from one leaf to another via the leaf traces. Necrosis of basal leaf vascular strands and of leaf traces of symptomless leaves adjacent to affected leaves can be seen in longitudinal sections of diseased plants. Roots and stems of more severely affected plants may show necrosis of vascular bundles extending from the leaf traces down through the stem and sometimes into the upper root, but generally root and inner stem tissues appear healthy. Further, necrosis in root vascular strands do not extend to the epidermal layers or to adventitious roots, indicating that infection from the soil does not take place via the root system, but rather from soil to leaves as also indicated by the occurrence of leaf lesions distant from the leaf base. Though no detailed histological examination was made it appeared that the pathogen, when found in stem and root, was restricted to the vascular system, since hyphae grew out from the necrotic vascular tissue but not from adjacent, healthy looking tissue. While numerous isolates of various *Pythium* spp. were obtained from the rhizoplane of tap roots of Chinese cabbage, none turned out to be *P. tracheiphilum*. Since in the previous year a 40–50% level of attack had been observed in the field from which roots were obtained, absence of the pathogen at the rhizoplane level also indicated that primary infection is hardly likely to take place via the root. It may be argued that the crop in question was not yet at the physiological stage of head formation at which attacks are normally observed. Thus, although infection by the root system cannot be completely ruled out, it seems obvious that soil to leaf infection is common and that plant colonization is primarily confined to the above ground parts. Possibly primary infection may also take place from soil via dying tissue of senescing first true leaves. At the time of head formation these have senesced and are in direct contact with the soil surface, and may thus offer a route of infection since they appear to rot off rather than to abscise. However, symptoms of infection have not been observed in such leaves. The onset of the disease closely coincides with the start of head formation. Attack of seedlings or of juvenile plants has never been observed, and plant emergence appears unaffected by the presence of the pathogen in the soil. Soil tends to accumulate in leaf sheathes and may also be a source for primary infection at leaf base level.

Discussion

The causal agent of a new disease of Chinese cabbage in Denmark has been identified as *Pythium tracheiphilum* Matta. This is the first record of *P. tracheiphilum* as a pathogen of Chinese cabbage.

The first disease outbreaks recorded in Denmark (in 1989) were initially interpreted as the result of a build up of inoculum of *Phytophthora porri* Foister in field soils, which at that time regularly and increasingly caused losses in Chinese cabbage in cold stores. The prevalence of this storage rot problem and a similarity of its symptoms to those caused by *P. tracheiphilum* led to the assumption that *Ph. porri* was the causal agent of the field disease. However, storage rot caused by *Ph. porri* progresses in a rather more uniform way from the base upwards in all leaves of the harvested head, whereas as discussed above, rot caused by *P. tracheiphilum* mainly progresses laterally, leaf by leaf, through the whorl into the heart. Furthermore, affected leaf material taken from the field has consistently yielded *P. tracheiphilum*, never *Ph. porri*. On the other hand isolations have been attempted from leaf material taken from heads rotted in stores. Using the above outlined isolation procedures, these attempts have consistently yielded *Ph. porri* and never *P. tracheiphilum*. Fagertun and Semb (1991) stated that in Norway *Ph. porri* is well known as a pathogen of stored white cabbage, swede, Chinese cabbage and cauliflower and that attacked plants may also be found in the field. It is not clear from their report, however, whether rot caused by *Ph. porri* in the field was specifically observed in Chinese cabbage.

There are no previous reports of pathogenic activity of *P. tracheiphilum* in crops in Denmark. However, a survey of the prevalence of oomycetes in nutrient solutions in Danish greenhouse ebb and flow systems in 1986 (Thinggaard and Middelboe, 1989) showed that *P. tracheiphilum* was present in Denmark.

Global records of the occurrence of *P. tracheiphilum* are few. It was first described as a vascular pathogen of lettuce in Italy in 1965, causing dwarfing and wilting of plants and blackening of vascular bundles of stems and roots (Matta, 1965). *P. tracheiphilum* has otherwise been reported attacking lettuce in the USA in 1974 (Tortolero and Sequiera, 1978), the Netherlands in 1975 (van der Plaats-Niterink, 1975), Germany in 1976 (Zinkernagel and Kröber, 1977, 1978), Canada in 1985 (Reeleder and Charbonneau, 1987), in England in 1988 (Hall, 1989) and in France (C. Alabouvette, pers. comm.). Unlike the European isolates of *P. tracheiphilum*, Wisconsin isolates were found to cause spreading, necrotic lesions on leaves of diseased lettuce, cultivar 'Minetto', for which reason the Wisconsin isolates were considered to constitute a new strain of the pathogen (Tortolero and Sequiera, 1978). In Sweden, *P. tracheiphilum* was isolated from spinach plants from 1 out of 28 fields examined and reported to be a relatively weak pathogen in that crop (Larsson, 1992). *P. tracheiphilum* has been reported to be responsible for damping off in pine nurseries in Algeria (Bhatnagar et al., 1993). In one case it has been isolated from the root of a rice seedling in poor vigour in Southern Australia (Cother and Gilbert, 1993), but pathogenicity was not confirmed. The fungus was isolated from irrigated soils but not from non-irrigated soils in the West Bank area of Gaza (Ali-Shtayeh, 1986).

Cultivation of Chinese cabbage has been practised for about 20 years in Denmark. The requirement of soil type and in particular of special farm equipment has led to the concentration of Chinese cabbage cultivation on a relatively small number of farms as well as to the practice of fairly short crop rotation schemes. As might be expected, this has led to an increase in diseases such as clubroot (*Plasmodiophora brassica*), and the development of the rot problem caused by *P. tracheiphilum* may likewise be a consequence of short rotation schemes. On the other hand, severe outbreaks of the disease have been observed in the southern Zealand on land never before cropped with Chinese cabbage. In one of these cases a crop of lettuce preceded the cabbage crop, and Chinese cabbage had been grown for some years in neighbouring fields of the same farm, indicating the possible importance of wind, water and machines in the spread of inoculum. It is noteworthy that dust storms are not uncommon in this area in the spring, when the soil surface is being prepared for sowing. Cultivation of lettuce possibly also contributes to the build up of inoculum in infested soil. *P. tracheiphilum* problems in Danish lettuce crops have not been reported and globally, records of the disease in lettuce are few which may indicate that the problem in this crop, where reported, was transient in nature. In Chinese cabbage, however, experience indicates that once the disease has become established on a farm, it reappears repeatedly in succeeding crops. As is often the case with soil-borne oomycetous species, persistence of *P. tracheiphilum* may be expected to exceed by far the duration of normal crop rotations. No clear-cut difference in degree of susceptibility between varieties (e.g. Kingdom 65, Storillo, Yoki) grown in

Denmark has been observed by us, but it is claimed by some farmers that they do notice a certain difference between varieties in this respect.

Because of its soil-borne nature, satisfactory control of the disease by fungicide application alone is unlikely. Thus cropping practices that may help in reducing disease incidence and sources of resistance need to be identified.

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