Pseudocercosporella capsellae, the cause of white leaf spot and grey stem of Cruciferae in Western Canada[1]

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White leaf spot and grey stem, a common disease of turnip rape ***(Brassica campestris)*** and rape (***B. napus)*** and several cruciferous weeds in Western Canada is described. The causal fungus is ***Pseudocercosporella capsellae.*** Formerly the stem symptoms on ***Brassica*** spp. were attributed to ***Mycosphaerella brassicicola,*** the cause of ring spot of B. ***oleracea,*** but a connection of this fungus with white leaf spot remains in doubt. Production of the phytotoxic red pigment "cercosporin" by ***P. capsellae*** is reported for the first time. Formation of thick-walled hyphae in stem tissue, often in the form of mycelial mats, is thought to represent an important adaptation permitting survival of the pathogen under adverse conditions.

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On décrit la tache blanche, maladie courante de la navette ***(Brassica campestris)*** et du colza ***(B. napus)*** et de plusieurs cruciferes adventices de l'ouest du Canada. L'agent de cette maladie est le champignon ***Pseudocercosporella capsellae.*** Auparavant, on attribuait les symptômes sur la tige des ***Brassica*** spp. a ***Mycosphaerella brassicicola,*** l'auteur de la tache annulaire de ***B. oleracea,*** mais un doute subsiste sur le rapport entre ce champignon et la tache blanche. On a d'autre part, signalé pour la premiere fois la production d'un pigment rouge phytotoxique "cercosporine" par ***P. capsellae.*** La formation d'hyphes a parois epaisses dans le tissu de la tige, souvent sous la forme de feutres myceliens, constituerait une importante adaptation permettant au pathogene de survivre en mauvaises conditions.

Introduction

In 1958, Vanterpool collected what appeared to be the disease ring spot ***[Mycosphaerella brassicicola*** (Fr. ex Duby) Lind.] on turnip rape ***(Brassica campestris*** L.) in east-central Saskatchewan (Vanterpool, 1960). However, ascocarps of ***M. brassicicola*** were never found on Cruciferae from the three Prairie Provinces at any time of the year and for many years spermogonia represented the only fruiting state observed. Within a few years of its discovery "ring spot" could be found late in the growing season throughout the park-belt of the prairies. Its appearance was often striking, particularly in northern park-belt areas where entire fields were discolored and no field free from it. Because of its late development, however, losses in yield appeared to be minimal.

Vanterpool subsequently observed a white leaf spot in fields of turnip rape and rape (***B. napus*** L.). Elsewhere in North America and abroad, white leaf spot has caused major crop losses, principally in white turnip (***B. rapa*** L.) (McKay, 1956; Miller and McWhorter, 1948). The pathogen involved was ***Pseudocercosporella capsellae*** (Ell. & Ev.) Deighton (Deighton, 1973). It will be shown in this paper that white leaf spot and grey stem ("ring spot") are different manifestations of a single disease. The earlier contention (Vanterpool, 1960) that the disease was ring spot now appears to be incorrect. It is our intention also, therefore, to examine points of similarity and difference between these two diseases.

Observations

Host ranges and symptoms of the white leaf spot and ring spot pathogens

We have found white leaf spot and grey stem in Saskatchewan on ***Brassica campestris,*** B. ***hirta*** Moench, ***B. napus,*** B. ***oleracea*** L. var. ***capitata*** L., B. ***oleracea*** L. var. ***botrytis*** L., B. ***napobrassica*** (L.) Mill., ***B. rapa, Capsella bursa-pastoria*** (L.) Medic., ***Conringia orientalis*** (L.) Dumort., and ***Neslia paniculata*** (L.) Desv. It has been reported on several other hosts (Deighton, 1973). ***B. oleracea*** is less severely affected than ***B. rapa*** or ***B. campestris*** and leaf symptoms on B. ***oleracea*** differ from those on the other two species (Miller and McWhorter, 1948). In contrast, ***M. brassicicola*** is largely restricted to oleraceous ***Brassica*** species (Dring, 1961; Weimer, 1926). Isolations made by the authors from the seed of turnip rape and from white leaf spots and grey stem lesions from cultivated and wild hosts from as far distant as Fort St. John, British Columbia, gave identical black, slow-growing colonies (Fig. 1F). White or buff-colored sectors were frequently observed in cultures of the wild-type from various sources. Conidia and spermogonia typical of ***P. capsellae*** were produced in culture, although in relatively small numbers.

Leaf spots produced by ***P. capsellae*** in nature are greyish white to brownish, often with a brown margin and occasionally with narrow line zonation (Fig. 1A, B).

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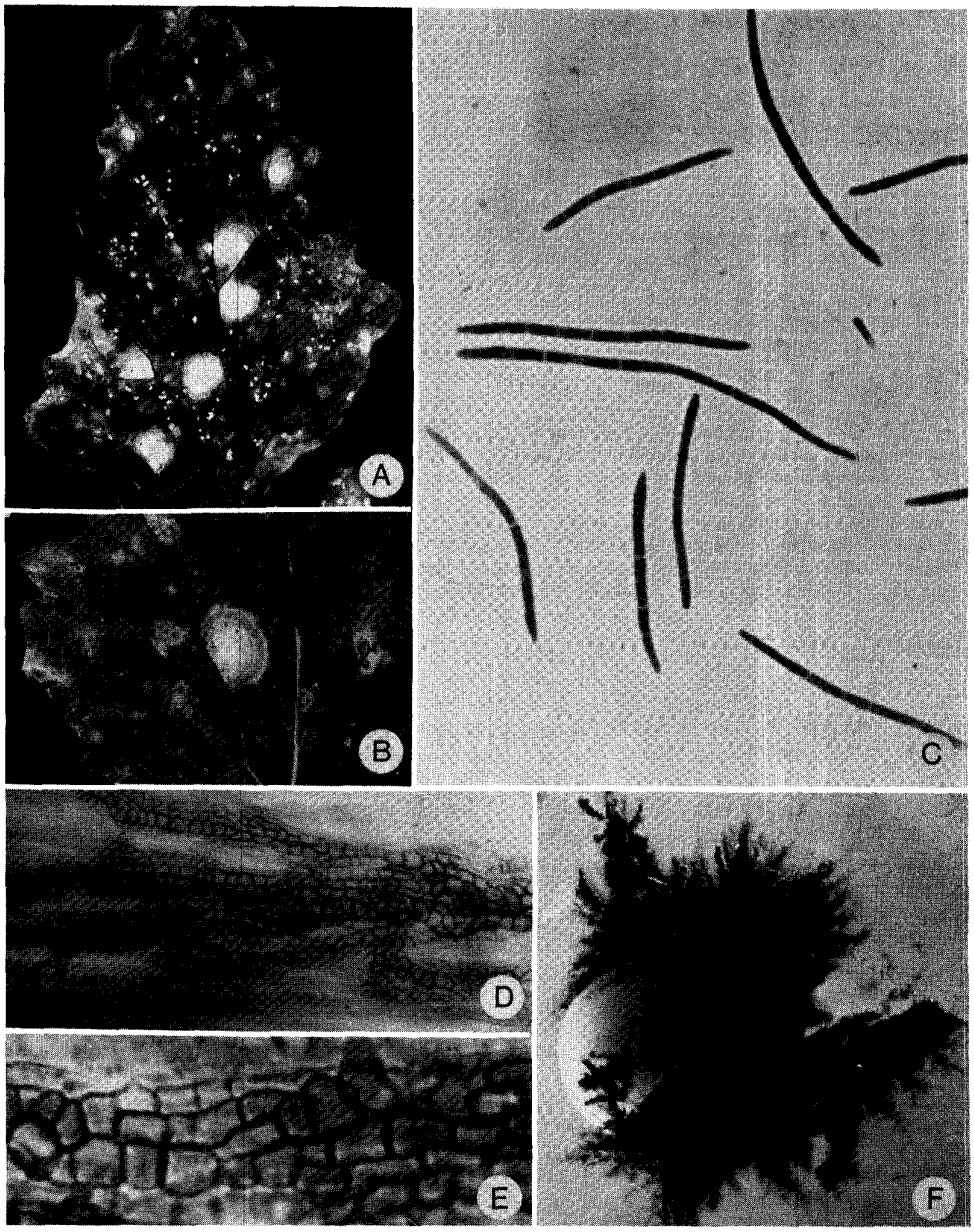

Figure 1. (A to F) *Pseudocercosporella capsellae,* the cause of white leaf spot and grey stem of rape. (A, B) Leaf symptoms from field collections of turnip rape and rape. Note narrow line zonation within spots. (C) Conidia of *P. capsellae* from leaf spots (natural infection). X 320. Spores stained with lactophenol-aniline blue. (D) Thick-walled overwintering mycelium of P. *capsellae* from a stem of turnip rape. Approx. X 100. (E) Enlargement of D. (F) Colony of *P. capsellae* growing from a naturally-infected seed of turnip rape.

McKay (1956) reported the presence of numerous "pseudo-sclerotia" embedded in infected leaf tissues. These were probably stromata, which vary considerably in size (Deighton, 1973). In comparison, spots caused by M. ***brassicicola*** on leaves have a brown background and contain black spermogonia, and frequently also perithecia, in a typical zonate arrangement. Each spot is surrounded by a narrow water-soaked area, with beyond this, a zone of chlorotic tissue. No fruiting bodies are formed when ringspot lesions are not exposed to moisture conditions near saturation (Nelson and Pound, 1959).

Mature stem and pod lesions caused by the two pathogens are similar in appearance. Transmission of ***P. capsellae*** in or on the seed itself seems to be of little consequence. Seed of ***Conringia orientalis*** from heavily infected pods failed to yield the fungus when plated on agar and seed of turnip rape and rape did so rarely (Fig. 1F). Infected bits of crop residue in seed samples may provide a ready means of transmission of the disease over wide distances. Such material is commonly found in samples of seed.

Morphology of P. capsellae and M. brassicicola

Those who have studied M. ***brassicicola*** are emphatic that only two spore stages occur, spermatial and ascosporic (McKay, 1956; Nelson and Pound, 1959; Snyder, 1946; Weimer, 1926). In ***P. capsellae*** a conidial and a spermatial state are found. This report would appear to be the first record of the latter for the white leaf spot pathogen. White leaf spots collected on ***Brassica*** spp. at Saskatoon in July during the past few years frequently gave no evidence of sporulation. Scores of conidia were obtained by incubating infected leaf pieces on wet filter paper for 24 h at 18-20°C under intermittent light.

Extensive immature brown lesions, along with mature greyish ones bearing numerous spermogonia, were collected on ***Capsella*** stems in late August, 1975. Stem segments bearing young lesions were incubated at 12 and 21°C under intermittent light in test tubes to which a small amount of water had been added. Within four days abundant ***Pseudocercosporella*** conidia formed at 12°C with only a few being obtained at the higher temperature. More mature stem lesions including some on ***B. campestris*** yielded few conidia under similar conditions.

Conidia from natural field infections ranged in length from 32 to 120 microns (mean 66) and were 2 to 3 microns wide. This agrees well with measurement recorded by others. Conidia from culture usually averaged about 5 microns longer than those from field material. Conidial morphology was as described by Deighton (1973). The presence of a truncate unthickened hilum on the conidium was an important characteristic serving to place the fungus under study in the genus ***Pseudocercosporella.*** The spores were usually 0- to 3-septate (Fig. 1C). Each cell was uninucleate and capable of germinating by a germ tube. Anastomoses of conidia via short germ tubes were observed. Infrequently segments of conidia from culture were seen to have developed into thick-walled cells. These might function as chlamydospores reminiscent of those formed in segments of spores in species of ***Fusarium***.

In Western Canada, ***P. capsellae*** overwinters on residues of crucifers in the form of dark, thick-walled, closely septate, matted hyphae (Fig. 1D, E) on which conidia are produced in the spring. These serve for primary dissemination of the disease. Subsequent spread is via secondary conidia formed on the primary lesions. Observations indicated that conidia may be carried down stems by coalescing dew drops or by rain drops, resulting in the elongate lesions so conspicuous in the autumn. These lesions often occur in series along the entire stem.

No ascosporic state of ***P. capsellae*** is known. In addition, despite an often concentrated search over many years, no authentic ascocarps of M. ***brassicicola*** have been found on Cruciferae in the Prairie Provinces since "ring spot" was first collected in this area in 1958 (Vanterpool, 1960). On several occasions a species of Mycosphaerella has been collected on crucifers and members of other families of dicotyledons in Saskatchewan and Alberta. This species has been identified as M. ***rassiana*** (de Not.) Johans. var. ***tassiana*** (Petrie and Vanterpool, 1978).

In the apparent absence of a perfect state the resistant thick-walled hyphae referred to earlier assume considerable importance in the survival of the pathogen during the extreme cold of the prairie winters and also, perhaps, during hot, dry periods in midsummer. The widespread occurrence of this stage of the life cycle on overwintering crop residue and to a lesser extent on weeds is an adaptation which permits primary infection to occur readily. Solel (1970) reported that mycelium of ***Cercospora beticola*** Sacc. survived for 3 years in beet leaf debris left on the soil surface under dry conditions. It is not known how long ***P. capsellae*** can survive in dead leaf or stem tissue.

Production of the pigment "cercosporin"

Several species of ***Cercospora*** and closely allied fungi produce the pigment "Cercosporin" (Lynch and Geoghegan, 1977). A red pigment was extracted from mycelial mats from ***P. capsellae*** cultures representing all of the collections of the fungus made to date by the authors. The material was soluble in ethanol, chloroform, ether and acetone but was insoluble in petroleum ether and in water. In concentrated sulfuric acid it was purple and in alkali, a distinctive green color. In 1N-NaOH the visible spectrum showed major absorption peaks at 480 and 645 mu and other minor peaks. It was toxic to rape, inhibiting seed germination and subsequent root growth. In all these respects it resembles cercosporin. An authentic culture of M. ***brassicicola*** obtained from Dr. P.H. Williams, University of Wisconsin, Madison, did

not produce detectible red pigment nor did it produce conidia of any type in culture. Dring (1961) reported the production of a toxin by M. ***brassicicola*** which acted in advance of the invading hyphae. This would appear not to be cercosporin, however. Cercosporin production by the white leaf spot pathogen has not been previously reported.

Discussion

Several pieces of evidence, many of which on their own are inconclusive, suggest that white leaf spot and grey stem has no connection with ring spot as found on the west coast of the United States and in Europe. It has been stated (Nelson and Pound, 1959) that M. ***brassicicola*** is strictly limited to cool, very moist regions but this is not totally convincing (McKay, 1956). Leaf symptoms would appear to be an obvious point of dissimilarity but this difference becomes much less marked when M. ***brassicicola*** develops under dry conditions. Other apparent differences include host range, nature of spores produced, apart from spermatia, and presence or absence of the pigment cercosporin. A more detailed comparative study of the two species under a range of environmental conditions is required.

In our work, inoculations of plants under controlled conditions gave inconsistent results. It is our view that development of a cultural technique enabling consistent production of large numbers of conidia should be accorded high priority at the beginning of such a study.

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