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Dark lines formed between colonies of isolates of Phoma exigua var. foveata on a semi-selective medium

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At the Research Institute for Plant Protection (IPO) in Wageningen, gangrene caused by the fungus *Phoma exigua* var. *foveata* is distinguished from other potato rots by plating small pieces of affected tissues on a semi-selective medium. On this medium the production of anthraquinones formed by *P. exigua* var. *foveata* is enhanced to such an extent that because of the presence of numerous yellow-green crystals colonies of this fungus can be differentiated easily from those of other ones growing out the same sample. The medium contains the following ingredients given as a.i. in 1 l of water: 10 mg Rose Bengal, 40 mg chlorothalonil, 300 mg metalaxyl, 250 mg streptomycin sulphate, 100 mg neomycin sulphate, 50 mg tetracycline chloride, 10 g Oxoid malt extract, and 15 g Oxoid agar No. 3.

In one of these tests dark lines were observed between some of the colonies. These lines differed from the violet lines commonly formed between colonies of the varieties *exigua* and *foveata* of *P. exigua* and which have been observed earlier (Langerfeld, 1973). Two types of isolates were obtained from colonies seperated by dark lines. These isolates again formed dark lines when mated to those of the other type but not when mated to isolates of the same type. Two representative isolates, one out of each type group, were designated type A (isolate 1707) and type B (isolate 1708). Experiments were initiated to find out if more types producing dark lines between colonies could be distinguished within the population of *P. exigua* var. *foveata* and *P. exigua* var. *exigua*. It was also studied wether the phenomenon was associated with cultural characteristics of isolates.

Isolates were mated by placing three to seven discs (4 mm) with mycelium of nine-day-old cultures grown on malt agar on the semi-selective medium in 9-cm petri dishes. In most cases one of the discs originated from a culture of a known dark-line type, the other ones from cultures of the isolate to be tested or of an isolate of known type. All cultures were incubated at 20 °C in the dark. Three different growth types of isolates were distinguished, viz. a mycelial, a pycnidial and an intermediate type. Isolates of the mycelial growth type did not form pycnidia on artificial media without prior exposure to NUV light, whereas those of the pycnidial and intermediate type, respectively, formed abundantly and scarcely pycnidia without prior exposure to NUV light.

Isolates differed with respect to the production of the antibiotic substance E dis-

covered by Logan and O'Neill (1970). The E+ type is marked by a local green staining of the medium after addition of a drop of NaOH. Most isolates of *P. exigua* var. *foveata* from The Netherlands do not form this antibiotic (Van Kesteren, 1975). In all tests three isolates of *P. exigua* were included.

In a first series of experiments five isolates of var. *foveata* were tested against type A and type B, and were mated to each other in all possible combinations. As can be seen in Fig. 1, isolate 1285 was similar to type A as no dark lines were formed between their colonies. Isolates 1255 and 1333 differed from type A and type B, and from each other as dark lines were formed between them. Isolate 1333 was of the pycnidial growth type, the others belonged to the mycelial growth type. This experiment yielded three new dark line types called type C (isolate 1255), D (isolate 1333) and E (isolate 1706).

In a second series, 27 isolates of the fungus were mated to the five different strains. Fifteen of these isolates showed dark lines to be formed with all colonies of type A, B, C, D and E, and consequently were of a different dark line type. In a third series, these 15 isolates were mated in all combinations. Amongst them six new types were found. In a fourth series, 25 isolates belonging to all 11 types were mated in all combinations in order to verify the results obtained in the foregoing series. The results confirmed that colonies formed only dark lines between them if they belonged to a different type.

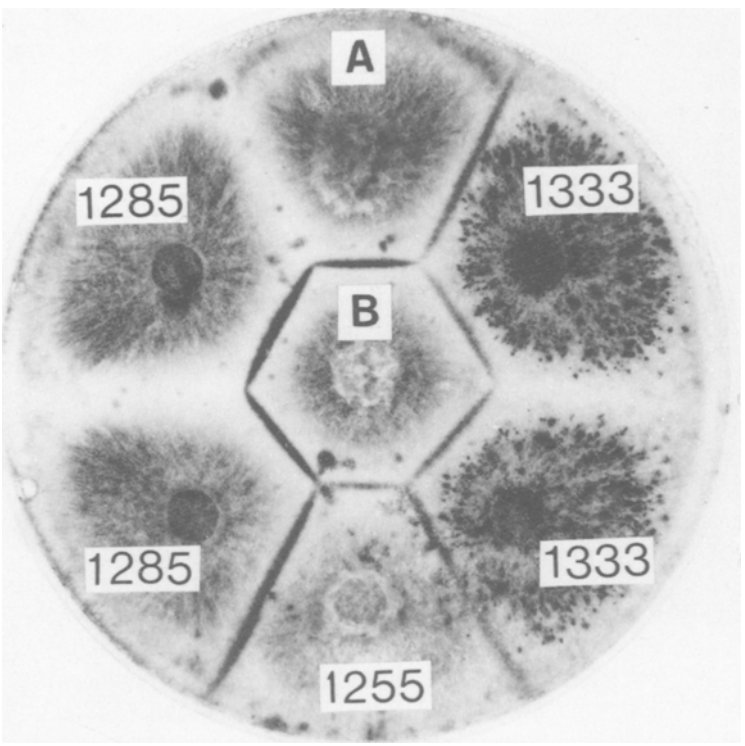

Fig. 1. Two-week-old colonies of isolates of *Phoma exigua* var. *foveata* tested against representatives of the dark line types A (isolate 1707) and B (isolate 1708).

Dark lines started to develop after the tenth day of incubation and after colonies had made contact. Violet lines between the two varities *exigua* and *foveata* started to develop on the fourth day without prior contact. No dark lines were formed between the two varieties. Unlike the violet lines the dark lines did not fade away and were not densely overgrown by the fungus. Mycelial growth ceased in the contact region and the area at both sides remained relatively hyaline compared to the darkening colonies. With time the 2-3 mm broad and sharply delimited lines turned totally black. The pigmented zone was superficial and did not penetrate deeper than 2 mm. In most cases dark lines were readily formed. However, isolate 1276 showed a different pattern. In combination with isolates of other types occasionally lines were not formed or were interrupted or short. This isolate is characterized by forming colonial sectors in which no anthraquinones are formed. Total or partial absence of dark lines was always associated with complete or local absence of anthraquinone crystals in colonies of isolate 1276.

Isolates of the dark-line type G and A were the most common ones. They were represented with nine and five isolates, respectively. Most types were represented with only one or two isolates. A direct relation between dark line type and mycelial growth type or E+ factor was not established.

Isolates are characterized by their dark line type. This feature may be useful in studies on population dynamics and it may offer a tool to study the combined effect of tuber-borne and soil-borne inoculum of *P. exigua* var *foveata* in a single experiment.

Samenvatting

De vorming van donkere lijnen tussen de kolonies van isolaten van Phoma exigua var. foveata op een semi-selectief medium

In het Instituut voor Plantenziektenkundig Onderzoek (IPO) te Wageningen wordt gangreen bij aardappel, veroorzaakt door de schimmel *Phoma exigua* var. *foveata*, onderscheiden van ander droogrot door aangetast weefsel uit te leggen op een semi-selectief medium. Op dit medium wordt de vorming van kristallen van voor de schimmel kenmerkende anthrachinonen bevorderd. De gemakkelijk waar te nemen geelgroene kristallen in het medium wijzen op de aanwezigheid van de betrokken schimmel. Bij een van deze toetsen vormden zich tussen de zich ontwikkelende kolonies van de schimmel donkere lijnen. Deze lijnen verschilden van reeds eerder beschreven violette lijnen, die zich vormen tussen kolonies van de twee variëteiten *exigua* en *foveata* van *P. exigua*. Onderzoek aan 27 isolaten van *P. exigua* var. *foveata* toonde aan dat er met betrekking tot de vorming van donkere lijnen 11 verschillende typen *P. exigua* var. *foveata* onderscheiden konden worden. Isolaten van deze typen vormden bij gepaarde groei op het selectieve medium donkere lijnen met andere typen, maar niet met isolaten van het zelfde type. Er bleek geen verband met het groeitype van de cultures noch met de vorming van het antibioticum 'E' te bestaan.

References

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