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Spread and survival of an aggressive and a non-aggressive strain of Ophiostoma ulmi in elms

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Abstract

Spread of an aggressive and a non-aggressive strain of *Ophiostoma ulmi* (Buisman) Nannf. in the susceptible elm *Ulmus hollandica* cl. Belgica was found to be similar after inoculation with a mixture of conidia of both strains. Survival of the aggressive strain in this clone was better than that of the non-aggressive strain. In *U. hollandica* cl. 390 and the 'Christine Buisman' elm, both resistant to the non-aggressive strain, spread of the non-aggressive strain was limited after inoculation of a mixture of conidia of both strains and the survival of the aggressive strain was also favoured over that of the non-aggressive one.

Additional keywords: Ulmus hollandica cl. Belgica, *U. hollandica* cl. 390, *U. carpinifolia* cl. Christine Buisman, bark beetles (aggressive strain *O. ulmi*).

Introduction

Since the introduction of the aggressive strain of *Ophiostoma ulmi* (Buisman) Nannf. into Britain, probably during the mid-1960's, Dutch elm disease has caused increasing losses of elm populations in that country (Gibbs, 1978). After the first discovery of an elm infected with the aggressive strain in the Netherlands in 1972 this strain spread rapidly through the entire country and it was isolated from about 50% of the samples collected from diseased trees in 1978. By now the aggressive strain has been recorded in many European countries and has caused an outbreak of the disease in the Casparian forests of Iran in 1977 (Afsharpour and Brasier, 1978).

As elm bark beetles are considered to be the vectors of the disease it is obvious that the elm bark beetle population must have become more and more contaminated with this aggressive strain of the pathogen during recent years. Not much is known, however, about the saprophytic part of the life cycle of the fungus in the bark of elms nor about its relation with the beetles. The observation by Gibbs and Smith (1978), that colonization of the inner bark by aggressive isolates appeared to be twice as fast as that by non-aggressive isolates may hold important implications for bark beetle contamination with the aggressive strain.

Although conclusive evidence is not available, it seems obvious that beetles can become carriers of the Dutch elm disease fungus after feeding in crotches of twigs of diseased trees or by making deep breeding galleries between wood and bark of diseased

trees. The latter activity also causes damage of the new annual ring which is inhabited by the pathogen. If the aggressive strain is favoured in spreading through the tree or has a better chance of survival in the tree than the non-aggressive one, it will be more frequently picked up by the beetle, thus causing an escalation of the disease.

In this study spread and survival of an aggressive and a non-aggressive strain were investigated in order to obtain more information about a possible favoured contamination of the beetles by the aggressive strain.

Material and methods

Seven-year-old nursery-grown callus cuttings of *Ulmus hollandica* cl. Belgica, susceptible to all strains of *O. ulmi* and of *U. hollandica* cl. 390, resistant to non-aggressive strains, were used. Twigs about 1 cm in diameter at the base were inoculated according to Elgersma (1969) on two sides with 30 µl of a mixture of suspensions of conidia of the aggressive strain H6 and the non-aggressive strain E2 in a ratio of 1:1, containing 6×10^5 viable conidia per ml. Viability was tested by plating out on Tchernoff's medium (Tchernoff, 1965). At various times after inoculation ten twig pieces of 3 cm in length were cut from between 0.5–3.5 cm (lower pieces) and ten from between 40–43 cm (upper pieces) above the site of inoculation. Each twig piece was cut into small pieces with a pair of pruning-shears and homogenized in 10 ml of sterile water for 1 min by means of an Ultra-Turrax homogenizer and then plated out in a dilution series. Colony counts were made after 3 days of incubation at 24 °C on Tchernoff's medium (Elgersma, 1969).

Similar experiments were performed using five-year-old *U. hollandica* cl. Belgica and *U. carpinifolia* cl. Christine Buisman trees of 1.5–2 m in height. Instead of twig inoculation the main stem of 30 trees of each clone was inoculated and twig pieces for homogenization were taken from the top part of the trees. Disease indices from 0–100 were determined according to Tchernoff (1965). Inoculations were performed as described before, but a mixture containing 27×10^5 viable conidia per ml of strain H6 and 39×10^5 viable conidia per ml of strain E2 was used.

Results

To compare the numbers of fungal propagules of the two strains in the wood-tissue, counted as colonies of either strain on the agar plate, a method was developed which made it possible to distinguish between colonies of the aggressive and the non-aggressive strain in an early stage of growth. A suspension of conidia or a homogenate of infected wood was mixed with the growth medium in a petri dish. After solidification of the agar a second layer of growth medium was poured on top. Three days after inoculation it was possible to distinguish between the two strains. Colonies of the non-aggressive strain showed up as discrete and compact colonies, in contrast to the colonies of the aggressive strain, which had a diffuse and less discrete appearance (Fig. 1). In Fig. 2 the numbers of propagules of an aggressive and a non-aggressive strain isolated from infected wood of *U. hollandica* cl. Belgica and *U. hollandica* cl 390 are compared at various times after inoculation. Results show that until 44 days after inoculation in clone Belgica the number of propagules in the homogenate appeared to be similar for both strains in the lower parts of the twigs as well as in the tops of twigs.

Fig. 1. Colonies of an aggressive (H6) and a non-aggressive strain (E2) of *O. ulmi* on agar plates. A) Colonies of strain E2. B) Colonies of strain H6. C) Colonies of both strains.

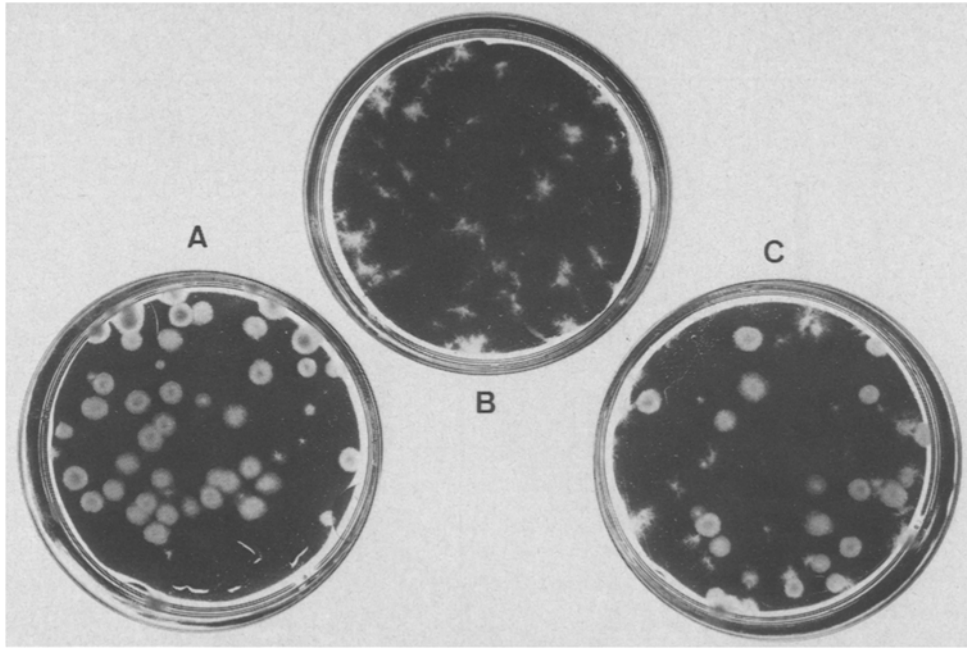

Fig. 1. Kolonies van een agressieve (H6) en een niet-agressieve stam (E2) van O. ulmi op agarschalen. A) Kolonies van stam E2. B) Kolonies van stam H6. C) Kolonies van beide stammen.

At this time twigs of clone Belgica were already dead. At 100 days after inoculation a significant difference showed up between the number of propagules isolated from each strain (Fig. 2a, b). Although both strains spread equally well throughout the twigs, the aggressive strain appeared to have a significantly ($p < 0.01$) better chance of survival in the tree than the non-aggressive strain.

In clone 390, resistant to the non-aggressive strain, growth of both strains appeared to be similar in the lower parts of the twigs at least until 10 days after inoculation. At later sampling times significantly more propagules of the aggressive strain could be isolated than from the non-aggressive one (Fig. 2c). In the upper twig pieces only small numbers of propagules could be isolated from the non-aggressive strain; however, propagules of the aggressive strain were isolated in great numbers (Fig. 2d). Twigs of clone 390 had not been killed even at 100 days after inoculation. We can therefore conclude that the infection of the upper twig parts was caused mainly by the aggressive strain.

Experiments with the susceptible clone Belgica and 5-year-old trees of the resistant clone Christine Buisman in which stem inoculations were performed and samples for homogenization were taken only from top parts, gave similar results (Fig. 3a, b). A disease index was also recorded in this case. Nine days after inoculation the disease index of clone Belgica was 38 and that of clone Christine Buisman 0; however, after 42 days the disease indexes had increased to 96 and 82, respectively.

Fig. 2. Colony counts after plating out of homogenates of 3 cm long twig pieces at various times after inoculation with a conidial suspension of an aggressive (H6) and a non-aggressive (E2) strain of *O. ulmi*. Data obtained at each sampling time were the average of 10 replicates. ■ aggressive strain; □ non-aggressive strain.

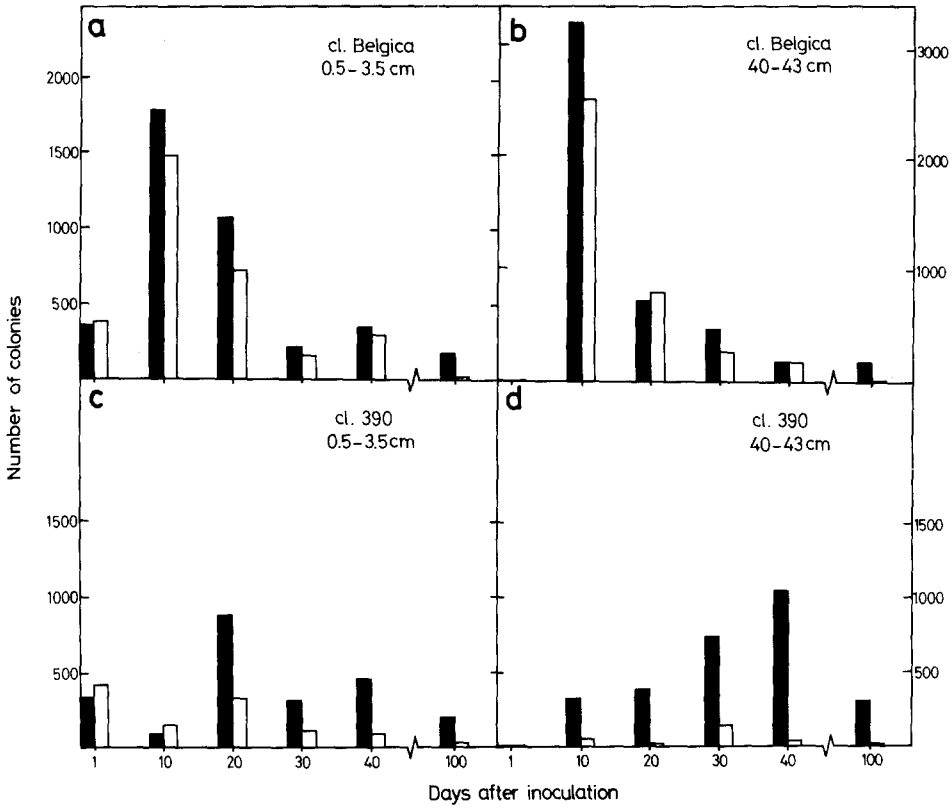

Fig. 2. Kolonietellingen na uitplaten van homogenaten van takstukjes van 3 cm op verschillende tijden na inoculatie met een conidiën-suspensie van een agressieve (H6) en een niet-agressieve (E2) stam van O. ulmi. De gegevens op ieder tijdstip van waarneming waren het gemiddelde van 10 herhalingen. ■ *agressieve stam;* □ *niet-agressieve stam.*

Discussion

If we presume that bark beetles can be contaminated by the Dutch elm disease fungus by feeding in crotches of infected twigs and that the fungus can also get into the beetle galleries which are made partly in the wood of infected trees, then contamination of beetles by the aggressive strain is enhanced by a prolonged survival of this strain in the tree. Especially elms which are more resistant to the disease, but which have a resistance mechanism towards the non-aggressive strain similar to that of clone 390 or Christine Buisman, will favour the spread of the aggressive strain relative to that of the non-aggressive one. The chance of survival is also less for the non-aggressive strain than for the aggressive one. These two factors would favour the chance of beetles becoming contaminated only by the aggressive strain in trees infected with both strains.

Fig. 3. Colony counts after plating out homogenates of stem pieces at 13 and 41 days after inoculation with a conidial suspension of an aggressive (H6) and a non-aggressive strain (E2) of *O. ulmi*. Data obtained at each sampling time were the average of 10 replicates. ■ aggressive strain; □ non-aggressive strain.

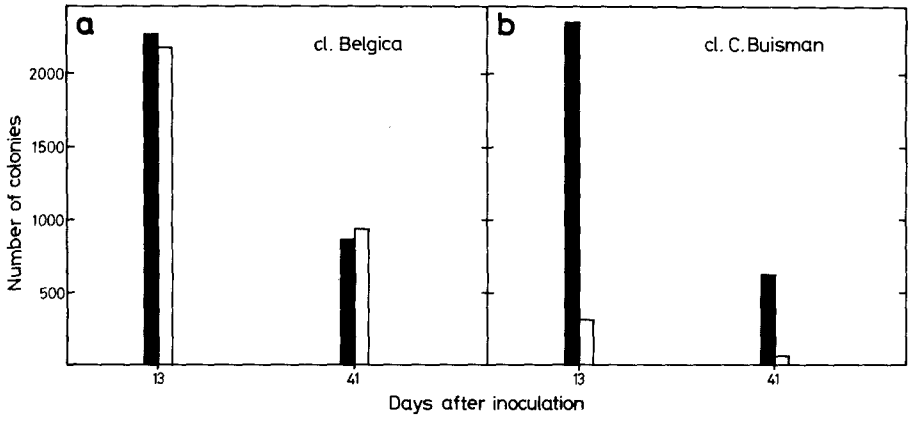

Fig. 3. Kolonietellingen na uitplaten van homogenaten van stamstukjes verzameld 13 en 41 dagen na inoculatie met een conidiënsuspensie van een agressieve (H6) en een niet-agressieve stam (E2) van O. ulmi. De gegevens op ieder tijdstip van waarneming waren het gemiddelde van 10 herhalingen. ■ agressieve stam; □ niet-agressieve stam.

Contrary to the findings of Gibbs et al. (1975), clone Christine Buisman is heavily affected by the aggressive strain in our experiments and therefore, the theory that this elm was selected for its resistance towards the aggressive strain in the early 1930's cannot be supported.

To understand the epidemiology of Dutch elm disease much more research has to be done about the relation between the beetle and the fungus, a field of research too long ignored by plant pathologists as well as entomologists.

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Samenvatting

Verspreiding en overleving van een agressieve en een niet-agressieve stam van Ophiostoma ulmi in iepen

De verspreiding van een agressieve en een niet-agressieve stam van *Ophiostoma ulmi* in de vatbare iep *Ulmus hollandica* kl. Belgica bleek vergelijkbaar te zijn na inoculatie met een mengsel van beide stammen. De overlevingskansen van de agressieve stam in deze iep bleken beter te zijn dan voor de niet-agressieve stam. In *U. hollandica* kl. 390 en in de 'Christine Buisman' iep, die beide resistent zijn tegen de niet-agressieve stam van het pathogeen, bleek de verspreiding van de niet-agressieve stam door de boom beperkt te zijn na een inoculatie met een mengsel van beide stammen. De overlevingskans van de agressieve stam was ook hier in beide klonen beter dan voor de niet-agressieve stam.

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