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Symptom expression in elms after inoculation with combination of an aggressive and a non-aggressive strain of Ophiostoma ulmi

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Spread of an aggressive and a non-aggressive strain of the Dutch elm disease fungus, *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, however, was better than that of the non-aggressive strain. In *U. hollandica* cl. 390 and in the Christine Buisman elm, both resistant to the non-aggressive strain, spread of the non-aggressive strain was limited after inoculation with a mixture of conidia of both strains and survival of the aggressive strain was better than that of the non-aggressive one (Elgersma and Heybroek, 1979).

The objective of this study was to observe and compare external symptom development in elms after inoculation with conidial suspensions of an aggressive and a non-aggressive strain of *O. ulmi* mixed in various ratios.

Seven-year-old nursery-grown callus cuttings of *U. hollandica* cl. Belgica, susceptible to all strains of *O. ulmi*, and of *U. hollandica* cl. 390, resistant to non-aggressive strains, were used.

Trees were inoculated according to procedures developed by Elgersma (1969) using mixed conidial suspensions of the non-aggressive strain E2 and the aggressive strain H6 in ratios of 4:1, 1:1 and 1:4. Twenty trees of each clone were inoculated with each mixture of conidial suspension. Ten trees of each clone were inoculated with conidia of the non-aggressive strain only and ten of each clone with the aggressive strain only. In all experiments, the total inoculum contained about 5×10^5 conidia per ml. Disease indices were based on external disease symptoms after Tschernoff (1965) and modified according to Elgersma (1969).

Clone Belgica showed heavy symptoms in all cases (Table 1). Leaf fall, however, was less severe in the trees inoculated with the non-aggressive strain only ($P < 0.002$) than in those inoculated with a mixture or with the aggressive strain only.

In clone 390, symptom expression was less severe when the inoculum contained more conidia of the non-aggressive strain. The data were subjected to analysis of variance and the Duncan's multiple range test. Plants inoculated with either aggressive or non-aggressive strains were not included in this statistical analysis, because only 10 plants had been inoculated. Symptom ratings taken 8 days after inoculation showed significant differences among all inoculum ratios tested. Effects of inoculum ratio 1:4 on elm as compared to ratio 1:1 and ratio 1:1 as compared to ratio 4:1 were significant at

Table 1. Disease indices of *U. hollandica* cl. Belgica and *U. hollandica* cl. 390 after inoculation with a mixture of conidial suspensions of a non-aggressive (E2) and an aggressive strain (H6) of *O. ulmi*. Figures between brackets represent the percentage of leaf fall.

Days after inoculation	Inoculum ratio non-aggr.: aggr.				
	non-aggr. only	4:1	1:1	1:4	aggr. only
	U. hollandica cl. Belgica				
8	55	50	47	60	60
11	95	94	85	100	100
63	80 (57)	90 (93)	89 (91)	98 (100)	100 (100)
	U. hollandica cl. 390				
8	1	4	7	14	19
11	1	12	20	42	49
63	3 (1)	6 (9)	21 (29)	74 (45)	95 (74)

Tabel 1. Ziekte-indices van U. hollandica kl. Belgica en U. hollandica kl. 390 na inoculatie met een mengsel van conidiënsuspensies van een niet-agressieve (E2) en een agressieve stam (H6) van O. ulmi. Getallen tussen haakjes geven het percentage bladval aan.

5% level. Effects of inoculum ratio 4:1 compared to ratio 1:4 were significant at 1% level. Ratings taken after 11 days showed a significant difference at 1% level between ratio 4:1 and 1:4 and between ratio 1:1 and 1:4. Differences between ratio 4:1 and 1:1 were not significant at either 5% or 1% level. Identical statistical results to the results of day 11 were found in ratings raken 63 days after inoculation.

The percentages of leaf fall after 63 days showed a significant (at 1% level) difference between treatment with inoculum ratio 4:1 and 1:4 and between ratio 4:1 and 1:1. A significant difference at 5% level was found between inoculum ratio 1:1 and 1:4. Apparently in this case ratings of percentage of leaf fall were differentiated better between the various treatments than recordings of disease indices.

Increases in symptom development after the first rating (8 days) were also statistically tested. Plants inoculated with ratios 4:1 or 1:1 showed no significant increase in symptom expression at 5% level. Plants inoculated with a ratio of 1:4 showed a significant increase (at 1% level) between 8 and 11 days and between 8 and 63 days of symptom development, but not between 11 and 63 days.

In control inoculation experiments with either 10^5 or 5×10^5 conidia of the aggressive strain no significant differences in disease expression could be observed. This is in accordance with the observation of Tchernoff (1965), who found no difference between 10^3 and 10^6 conidia per ml in the development of symptoms of the disease in susceptible elms after inoculation with conidial suspensions. The lower disease indices obtained after inoculation with mixtures of conidial suspensions indicate a suppression of symptom development related to the ratio of conidia of the non-aggressive and the aggressive strain in the inoculum.

It seems that the non-aggressive strain induces a mechanism in the resistant elm tree

which counteracts at least partially the induction or expression of disease symptoms provoked by the aggressive strain of *O. ulmi*.

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Samenvatting

Symptoomexpressie in iepen na inoculatie met diverse combinaties van een agressieve en een niet-agressieve stam van Ophiostoma ulmi

De vatbare iep *Ulmus hollandica* kl. Belgica en de voor de niet-agressieve stam resistente iep *U. hollandica* kl. 390 werden geïnoculeerd met mengsels van conidia van een agressieve en een niet-agressieve stam van *Ophiostoma ulmi*. Kloon Belgica vertoonde in alle gevallen ernstige symptomen, maar bij kloon 390 werd een geringere symptoomontwikkeling waargenomen naarmate de hoeveelheid conidia van de niet-agressieve stam in het mengsel toenam. Kennelijk induceert de niet-agressieve stam een mechanisme in deze plant, dat althans gedeeltelijk de inductie of expressie van ziektesymptomen veroorzaakt door de agressieve stam, tegengaat.

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