Neth. J. Pl. Path. 82 (1976) 161–172

Production of pectic and cellulolytic enzymes by aggressive and non-aggressive strains of Ophiostoma ulmi

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Accepted 13 May 1976

Abstract

Polygalacturonase (PG) and cellulase (Cx) production by *Ophiostoma ulmi* was determined in growth media of pH 3.0, 5.0 and 7.0 containing ethanol-extracted or freeze-dried elm wood. PG activity was low or absent in cultures of pH 7.0. Cx production was not affected by differences in pH. Production of PG was much higher with freeze-dried wood than with ethanol-extracted wood. No differences in PG and Cx activities were found between cultures with freeze-dried wood of resistant or of susceptible elms. No correlation was found between PG and Cx production and aggressiveness of strains of *O. ulmi*. The decrease of PG activity during incubation with increasing pH, was not due to the formation of PG inhibitors.

Introduction

The in vitro production of cell wall degrading enzymes by *Ophiostoma ulmi* (Buisman) Nannf. has been reported by several investigators (Beckman, 1956; Husain and Dimond, 1958; Holmes et al., 1959; Biehn and Dimond, 1971). These enzymes may play an important rôle in the development of symptoms of the disease by releasing partially hydrolyzed polysaccharides of the host cell walls in the vessel lumina thus obstructing water transport (Beckman, 1956; Gagnon, 1967; Dimond, 1972).

Recently, an aggressive strain of *O. ulmi*, with recognizable cultural characteristics (Gibbs and Brasier, 1973), has caused serious damage to the elm population in Britain (Gibbs et al., 1972). Even some of the hitherto 'resistant' elm clones are susceptible to this strain (Gibbs et al., 1975; Heybroek, 1975).

Scanning electron microscope studies showed that the aggressive strain develops more rapidly in the 'resistant' elm, *Ulmus hollandica* cl. 390, than does the non-aggressive strain (Miller and Elgersma, 1976). Obviously, the resistance mechanisms as described by Elgersma (1970, 1973) do not operate effectively enough to limit the spread of the aggressive strain. An explanation might be that the aggressive strain produces cell wall degrading enzymes faster than does the non-aggressive strain, thus enabling the former to bypass vessels with tyloses and gum. Therefore, in the present study the production of pectic and cellulolytic enzymes by aggressive and non-aggressive strains was compared.

Materials and methods

Growth medium. Enzyme production by *O. ulmi* was examined in shake cultures at 20°C in 200 ml Erlenmeyer flasks containing 40 ml of a growth medium. Media were buffered at either pH 7.0, 5.0 or 3.0 using 0.02, 0.1 and 0.2 M K_2HPO_4–KH_2PO_4 buffers. A pH 3.0 was obtained by adding concentrated phosphoric acid to a solution of KH_2PO_4. The buffer solutions were supplemented with 2 g asparagine, 10 mg $FeCl_3$, 1 mg thiamine and 1 mg pyridoxine per liter. As carbon source 0.4 g ethanol-extracted or freeze-dried ground elm wood per flask was used.

First year elm shoots of various clones of *U. hollandica* and *U. americana* were collected during July. The bark was stripped off carefully, the remaining wood dipped into a 0.1 M phosphate buffer pH 5.5, containing 0.2% sodium ascorbate and 2 mM cysteine, chopped into small pieces and immediately dropped into liquid nitrogen. The frozen elm wood was either freeze-dried or, according to Biehn and Dimond (1971), boiled in 96% ethanol, 10 ml per g fresh weight, for 1 hour, filtered and resuspended in fresh 96% ethanol. Ethanol was refreshed after 1, 3 and 5 days. After 1 week, the elm wood was filtered and dried in an oven at 60°C. The freeze-dried and the ethanol-extracted elm wood were ground to pass a 0.5 mm screen in a Culatti micromill. The elm wood was sterilized by keeping it at 80–90°C for at least 48 hours.

Inoculum. Conidia of *O. ulmi* were produced in shake culture on Tchernoff's medium (Tchernoff, 1965) at 20°C for 4 days. Mycelium was removed by filtering through glasswool. Conidia were washed 3 times in sterile distilled water and the concentration of conidia was adjusted to 0.4×10^8 conidia per ml with the aid of a haemocytometer. Five ml of this suspension was used for inoculation of each flask containing 40 ml of medium. The aggressive strains H6 and W4 (code of strains according to Gibbs et al., 1975) and the non-aggressive strains TXM/3 (Tchernoff, 1965) and S2, collected by A. N. Copijn, Bros Copijn Limited, in 1973, were used. In addition to these strains, the strain TX51 collected by H. M. Heybroek in Kashmir, India, in 1960, was included.

Enzyme preparation. At various intervals after inoculation, cultures were centrifuged to remove elm wood and fungal material, filtered through a Whatman no 6 filter paper and a Sartorius membrane filter (pore size 1.2 μm). Twenty-five ml of the culture filtrate from each flask was saturated with ammonium sulphate and, after being allowed to stand for 24 hours at 4°C, subsequently centrifuged. The precipitate was dissolved in 3 ml 0.1 M acetate buffer, pH 5.5 and used as a crude enzyme preparation.

Enzyme assays. Polygalacturonase (PG) and cellulase (Cx) activities were assayed either by measuring increase in reducing groups, or reduction in viscosity of the substrates. The reaction mixtures for PG activity measurements contained 8 ml solution of 1.7% sodium polypectate and 1% ammonium oxalate, 4 ml 0.2 M acetate buffer solution of pH 4.5 and 1 ml crude enzyme preparation. The reaction mixtures for measuring Cx activity contained 8 ml solution of 1% carboxymethylcellulose, 4 ml 0.2 M phosphate buffer of pH 7.0 and 1 ml crude enzyme solution. The reaction

mixtures were incubated at 30 °C. The release of reducing groups was determined after Nelson (1944). Viscometric determinations were performed in Ubbelohde ASTM no 4 viscometers. Activities were expressed as relative viscometric units (RVU), defined as 1000 × the reciprocal of time (min) for a 50% decrease in relative viscosity of the reaction mixture. Samples with activities above 200 RVU were diluted for more precise activity determinations.

Pectate lyase activity was assayed by the thiobarbituric acid reaction according to Albersheim et al. (1960) using the reaction mixture as described for PG activity measurements, as well as a reaction mixture of 12 ml of a 1% solution of sodium polypectate in 0.02 M Tris, pH 8.5, and 1 ml of enzyme preparation.

Thin layer chromatography. In order to demonstrate galacturonic acid or glucose as the end products of the enzymatic degradation of the polymers, the reaction mixtures, to which a few drops of toluene were added, were incubated for 24 hours at 30 °C. Ethanol was added until the reaction mixtures contained 70% of ethanol. After centrifugation the supernatants were dried by means of vacuum evaporation with slight heating (less than 40 °C) and redissolved in 0.5 ml of distilled water; 25 µl of these solutions were spotted on precoated silica gel TLC plates (layer thickness 0.25 mm) and developed in ethylacetate – acetic acid – water (3:3:1). The plates were sprayed with the naphthoresorcinol-sulphuric acid reagents and heated for 5–10 min at 105 °C.

Growth of the fungus during incubation. Increase of fungal propagules in the culture medium during incubation was determined at various intervals by culturing appropriately diluted samples on Tchernoff's medium containing 1.3% of agar. The length of 50 germ tubes was measured after 26 hours of incubation.

Disease indices. For each strain of *O. ulmi* tested, ten 7-year old elms of the 'resistant' clone 390 were inoculated with a suspension of 5×10^6 conidia per ml. Inoculations were performed in the second week of June. Disease indices were based on the external disease effects and estimated according to Tchernoff (1965).

Experiments and results

Cultural conditions of pectic and cellulolytic enzyme production. The cultural conditions favourable for pectic and cellulolytic enzyme production were studied by growing the non-aggressive strain TXM/3 in media kept at various pH by buffers of varying molarity. Enzyme activities, numbers of propagules, length of germ tubes and final pH were determined. The fungus was grown either on ethanol-extracted wood (Table 1) or freeze-dried wood (Table 2). PG activity was much higher when the fungus was grown on freeze-dried wood than on ethanol-extracted wood, especially when the initial pH was low. In both experiments, using freeze-dried and ethanol-extracted wood, the highest PG activity was attained in a 0.02 M buffer of pH 3.0, after 26 hours. This activity was 7 to 8 times higher when the fungus was grown on freeze-dried than on ethanol-extracted wood. PG activity was either low or negative in cultures grown at pH 7.0 or when the pH became even higher during incubation as in cultures with weak buffering capacities. Media with a relatively high ionic strength

Table 1. PG and Cx activities, number of propagules, length of germ tubes and final pH measured at various intervals after inoculation of the growth medium with *O. ulmi*, strain TXM/3. The fungus was grown on ethanol-extracted wood.

Molarity of buffer	Initial pH	Time in hours after inoculation												
		26					68				140			
		PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	length germ tubes (μm)	final pH	PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	final pH	PG (RVU)	Cx (RVU) ($\times 10^5$)	propagules ($\times 10^5$)	final pH
0.02	7.0	4	8	144	55	7.1	6	16	278	7.2	5	49	514	8.6
0.1	7.0	±[1]	3	87	55	7.1	0	7	208	7.1	0	12	305	7.5
0.2	7.0	+	+	70	50	7.0	0	4	143	7.1	0	6	180	7.1
0.02	5.0	56	33	381	165	7.1	5	63	620	8.2	0	63	575	8.4
0.1	5.0	37	17	157	226	6.2	59	40	425	6.4	77	46	440	6.5
0.2	5.0	36	10	89	220	5.9	35	32	311	6.1	37	31	297	6.0
0.02	3.0	133	20	183	116	6.2	59	63	781	7.5	10	57	624	7.7
0.1	3.0	18	4	67	127	3.5	65	35	412	6.0	87	44	465	6.0
0.2	3.0	4	+	42	104	3.3	39	7	200	4.5	67	8	234	4.6

[1] ± very low activity.

Tabel 1. PG- en Cx-activiteit, aantal propagula, kiembuislengte en eind-pH gemeten op verschillende tijdstippen na inoculatie van het kweekmedium met O. ulmi, stam TXM/3. De schimmel werd op met ethanol geëxtraheerd hout gekweekt.

Table 2. PG and Cx activities, number of propagules, length of germ tubes and final pH measured at various intervals after inoculation of the growth medium with *O. ulmi*, strain TXM/3. The fungus was grown using freeze-dried wood.

Molarity of buffer	Initial pH	Time in hours after inoculation												
		26					68				140			
		PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	length germ tubes (μm)	final pH	PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	final pH	PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	final pH
0.02	7.0	9	13	176	95	7.2	20	36	402	7.4	+	100	651	8.7
0.1	7.0	+[1]	+	58	79	7.0	+	16	200	7.0	8	39	332	7.2
0.2	7.0	+	+	38	79	7.0	+	9	133	7.0	+	28	167	7.2
0.02	5.0	231	42	227	300	7.8	0	48	225	8.5	0	51	158	8.3
0.1	5.0	209	15	78	294	6.5	258	33	254	6.4	271	36	262	6.4
0.2	5.0	121	10	40	274	5.9	190	20	136	6.0	266	27	167	6.2
0.02	3.0	1023	31	248	257	6.7	148	65	435	7.3	+	67	566	7.3
0.1	3.0	532	13	76	304	5.1	709	33	270	5.7	759	44	384	6.0
0.2	3.0	133	17	36	193	3.5	543	14	144	4.4	633	18	320	4.7

[1] + = very low activity.

Tabel 1. PG- en Cx-activiteit, aantal propagula, kiembuislengte en eind-pH gemeten op verschillende tijdstippen na inoculatie van het kweekmedium met O. ulmi, stam TXM/3. De schimmel werd op gevriesdroogd hout gekweekt.

(0.2 M) showed less PG activity compared to media with a lower ionic strength (0.1 M). This was probably due to the fact that growth was limited at high ionic strength. This phenomenon was even more pronounced with Cx.

The use of freeze-dried or ethanol-extracted wood as carbon source did not seem to have much effect on Cx activity. Cx activity tended rather to be correlated with the number of propagules. The number of propagules in the media immediately after inoculation was about 27×10^5 per ml in both experiments. In general the increase in propagule numbers showed the same pattern in both experiments. Growth increase was highest in the medium of low ionic strength and lowest at a relatively high ionic strength (0.2 M).

Growth of germ tubes was significantly reduced in media of pH 7.0 ($p < 0.01$, Wilcoxon two sample test) compared to media of pH 5.0 and 3.0.

Production of pectic and cellolytic enzymes on wood of resistant trees. The possibility that resistant trees may contain substances with inhibitory effect on enzyme production or growth of the pathogen was studied by adding conidia of strain TXM/3 to a 0.02 M medium of pH 3.0 and using freeze-dried wood collected from the resistant elm clones 543, 296, 390, Chr. Buisman and the susceptible elm *U. americana* as carbon source. Table 3 shows that enzyme activities and growth of the fungus were not inhibited when using wood of resistant elms instead of wood of a susceptible elm.

Production of pectic and cellulolytic enzymes by aggressive and non-aggressive strains. To verify our hypothesis of a higher rate of production of cell wall degrading enzymes by aggressive strains compared to non-aggressive ones, PG and Cx activities and growth of strains of *O. ulmi* were determined at various intervals after inoculation. The strain TX51 was also included, because this strain caused a disease expression

Table 3. PG and Cx activities, number of propagules, length of germ tubes and final pH measured at various intervals after inoculation of the growth medium with *O. ulmi*, strain TXM/3. The fungus was grown using freeze-dried wood of resistant and susceptible elms.

Elm clone	26 hours after inoculation				
	PG (RVU)	Cx (RVU)	propagules ($\times 10^5$)	length germ tubes (μm)	final pH
cl. 543[1]	782	15	189	251	6.8
cl. 296[1]	665	18	188	234	6.7
cl. 390[1]	831	20	208	201	6.9
cl. Chr. Buisman[1]	451	14	167	263	7.1
U. americana[2]	618	14	201	211	6.2

[1] Resistant.
[2] Susceptible.

Tabel 3. PG- en Cx-activiteiten, aantal propagula, lengte van kiembuizen en eind-pH gemeten op verschillende tijdstippen na inoculatie van het kweekmedium met O. ulmi, stam TXM/3. De schimmel werd gekweekt op gevriesdroogd hout van resistente en vatbare iepen.

Table 4. PG and Cx activities, number of propagules, length of germ tubes and final pH measured at various intervals after inoculation of the growth medium with various strains of *O. ulmi*. The fungus was grown using freeze-dried wood.

Strain of *O. ulmi*	Time in hours after inoculation															
	26					44				68				140			
	PG (RVU)	Cx (RVU)	propa-gules ($\times 10^5$)	length germ tubes (μm)	final pH	PG (RVU)	Cx (RVU)	propa-gules ($\times 10^5$)	final pH	PG (RVU)	Cx (RVU)	propa-gules ($\times 10^5$)	final pH	PG (RVU)	Cx (RVU)	propa-gules ($\times 10^5$)	final pH
H6[2]	398	14	150	136	6.6	183	86	895	7.4	75	89	965	7.5	0	95	1077	7.4
W4[2]	166	24	171	195	6.5	99	111	668	7.5	12	121	720	7.5	0	111	922	7.5
S2	156	95	328	206	7.0	23	202	798	7.5	+	253	835	7.6	0	296	1455	7.6
TXM/3	690	33	256	193	6.8	183	60	412	7.5	44	48	592	7.6	0	86	727	7.6
TX51	+[1]	+	63	303	7.1	0	26	72	7.5	0	23	87	7.5	0	30	76	7.6

[1] + — very low activity.
[2] Aggressive strains.

Tabel 4. PG- en Cx-activiteiten, aantal propagula, lengte van kiembuizen en eind-pH gemeten op verschillende tijdstippen na inoculatie van het kweekmedium met O. ulmi. De schimmel werd op gevriesdroogd hout gekweekt.

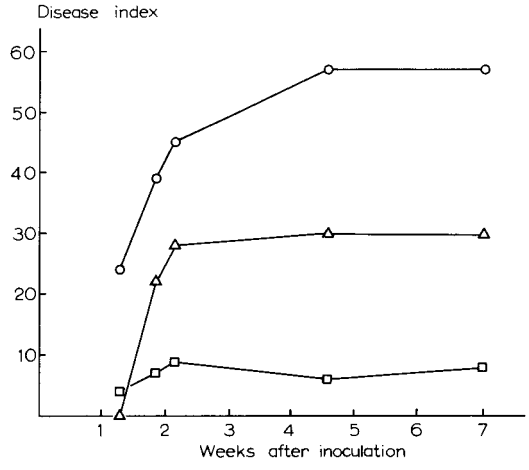

Fig. 1. Disease index of various strains of *O. ulmi* ○——○ H6 (aggressive), △——△ TX51 and □——□ S2 (non-aggressive).

Fig. 1. Ziekte-index van verschillende stammen van O. Ulmi. ○——○ *H6 (agressief),* △——△ *TX51 en* □——□ *S2 (niet-agressief).*

rating between the aggressive and the non-aggressive strain (Fig. 1). The 0.02 M medium of pH 3.0 was selected to incubate these strains, and freeze-dried wood collected from the susceptible elm, *U. hollandica* cl. Belgica, was used as carbon source. Results are given in Table 4. Strain TXM/3 showed the highest PG activity and strain S2 gave the highest Cx activity after 26 hours of incubation. There was no correlation between PG or Cx activity and aggressiveness of the strains. Surprisingly, the strain collected in India, TX51, showed, in contrast to all other strains tested, a very low PG activity or no activity at all under conditions, which appeared to be very favourable for PG production. The number of propagules measured during incubation was relatively small for TX51, but germ tubes were significantly longer after 26 hours of incubation ($p < 0.01$, Wilcoxon two sample test) than those measured for all other strains. The pH increased and PG activity decreased during incubation for strain TXM/3 as described earlier.

Disease assessment caused by the aggressive strain H6, the non-aggressive strain S2 and strain TX51 are shown in Fig. 1. As was expected, strain H6 caused severe symptoms in contrast to the non-aggressive strain. Strain TX51 was more virulent than the non-aggressive strain, but was not as aggressive as strain H6. The first symptoms appeared somewhat later after inoculation with TX51 than after inoculation with the aggressive and non-aggressive strains.

Decrease of PG activity at relatively high pH. The observed correlation between a relatively high pH (above 7) and a low PG activity, or a complete absence of PG activity may well be due to the formation of PG inhibitors, e.g. oxidation products of phenols. In order to investigate this aspect 15 ml of a sterile culture filtrate of pH 6.2, obtained from 0.02 M buffered medium of pH 3.0, inoculated with strain TXM/3 and incubated for 26 hours, was mixed with:

a) 25 ml of 0.1 M phosphate buffer, pH 8.0,
b) as a, plus 0.4 g of freeze-dried wood,
c) as b, plus 1 ml of 2×10^8 conidia per ml,
d) 25 ml of 0.1 M phosphate buffer, pH 6.0,
e) 25 ml of sterile distilled water.

Table 5. PG activity of a mixture of 15 ml culture filtrate of *O. ulmi*, strain TXM/3 and 25 ml buffer of pH 8.0 or pH 6.0 with or without addition of freeze-dried wood or freeze-dried wood and conidia. PG activity was assayed immediately after mixing or after 24 hours of incubation on a shaker at 20°C.

Composition of the solution or suspension added to the culture filtrate	PG activity (RVU)		
	assayed after 24 hours	assayed immediately after mixing	final pH
0.1 M buffer, pH 8.0	56	118	7.6
0.1 M buffer, pH 8.0 and 0.4 g wood	91		7.6
0.1 M buffer, pH 8.0, 0.4 g wood and 2.10^8 conidia	125		7.6
0.1 M buffer, pH 6.0	47	108	6.0
distilled water	0	82	6.2

Tabel 5. PG-activiteit van mengsels van 15 ml cultuurfiltraat van O. ulmi, stam TXM/3 en 25 ml buffer-oplossing van pH 8,0 of pH 6,0 met of zonder toevoeging van gevriesdroogd hout of gevriesdroogd hout en conidiën. PG-activiteit werd bepaald direct na mengen of na 24 uur incubatie bij 20°C in een schud-machine.

All buffer solutions used were sterilized and contained asparagine, vitamins, and $FeCl_3$, as described in 'Materials and methods'. The mixtures were incubated for 24 hours on a shaker at 20°C and the PG activity was assayed. Enzyme activities in the mixtures as described under a, d and e were also determined immediately after mixing. Results(Table 5)show a decrease in enzyme activity after shaking the reaction mixtures for 24 hours at 20°C, except when conidia were added. PG had been inactivated for about 50% of its activity during this period when incubated with either a buffer of pH 8.0 or a buffer of pH 6.0. Obviously these differences in pH of the reaction mixture do not affect the rate of inactivation of the enzyme. Addition of freeze-dried wood even hampered the rate of inactivation of PG and when conidia were added to this reaction mixture no decrease of enzyme activity could be found. This was probably due to the fact that PG synthesis in this case had compensated the loss in PG activity by inactivation. When distilled water was added instead of a buffer solution, it appeared that PG was completely inactivated after 24 hours at 20°C. It may be concluded, therefore, that no PG inhibitors are released from the freeze-dried wood during incubation and that the decrease in PG activity may be explained solely by an inhibited synthesis of PG in a medium with a relatively high pH compared to a medium with a relatively low pH. To verify this conclusion 0.1 M buffer solutions of either pH 7.5 or pH 5.5 to which 0.2% sodium polypectate had been added as carbon source were inoculated and the enzyme activity was assayed after 48 hours of incubation. The enzyme activity of the culture with initial pH 5.5 appeared to be 17 RVU and no PG activity was assayed in a culture with initial pH 7.5. These results are in accordance with the above conclusion.

Some characteristics of the pectic and cellulolytic enzymes produced by aggressive and non-aggressive strains. Crude enzyme preparations obtained from an aggressive and a non-aggressive strain of *O. ulmi* caused a 50% decrease in viscosity with only about 0.2% hydrolysis of the sodium polypectate. Galacturonic acid could be detected by means of TLC when sodium polypectate was incubated with the crude enzyme prepa-

ration. Products of hydrolysis did not react with the thiobarbituric acid reagents. Evidently the aggressive and non-aggressive strains produce an endopolygalacturonase and no pectate lyase.

In contrast to Husain and Dimond (1958) we were not able to detect glucose as a reaction product of Cx activity after 24 hours of incubation with an enzyme preparation of relatively high activity (200 RVU).

Discussion

Isolation of pectic and cellulolytic enzymes from infected elm wood has not been succesful (Elgersma, 1969). Woods and Holmes (1974) were only able to obtain enzyme preparations of rather low PG activity.

However, differences in distribution patterns of aggressive and non-aggressive strains in infected trees may be expected, which makes a comparison of enzyme production in vivo rather ambiguous.

The fungus was therefore grown in vitro on ground elm wood as carbon source. Freeze-dried wood induced a much higher PG production than ethanol-extracted wood, as used by Biehn and Dimond (1971). Probably PG-inducing substances had been removed by this treatment. Consequently, in comparing enzyme production of isolates differing in aggressiveness only freeze-dried elm wood was used. Wood collected from resistant elms instead of susceptible trees had no apparent influence on PG or Cx activity, or growth of the pathogen. The in vitro experiments did not show a correlation between aggressiveness and PG or Cx activity. It still remains uncertain if production rates of these enzymes in vitro are comparable to the production rates in vivo. Strain TX51, however, which caused a disease expression rating between that of the aggressive and of the non-aggressive strain, produced only negligible amounts of PG, or no PG at all, under conditions which enabled other strains to produce considerable amounts of PG. Therefore the rôle of PG in symptom expression is rather doubtful, if assuming that the mechanism by which TX51 induces symptoms is the same as for other strains of *O. ulmi*. Disease symptoms appeared a few days later in 'resistant' trees inoculated with strain TX51 than in 'resistant' trees inoculated with other strains (Fig. 1). This was also observed in case susceptible trees were inoculated. The rate of conidia formation in vitro by TX51 was rather low (Table 4). If conidia are formed at a low rate in infected trees we may expect a slower distribution of the fungus and consequently a somewhat delayed disease development. But a lower rate of conidia formation does in this case not imply a lower level of virulence. This is in contrast to what would be expected, since a slow distribution rate of the fungus enables the resistance mechanism of the tree to be more effective (Elgersma, 1973.

Samenvatting

Produktie van pectolytische en cellulolytische enzymen door agressieve en niet-agressieve stammen van Ophiostoma ulmi

Produktie van polygalacturonase (PG) en cellulase (Cx) door *Ophiostoma ulmi* werd bepaald in media van pH 3,0, 5,0 en 7,0 met in ethanol geëxtraheerd (Tabel 1) of met

gevriesdroogd iepehout (Tabel 2) als koolstofbron. In culturen bij pH 7,0 was de PG-activiteit laag of niet aantoonbaar. Cx-produktie werd niet door de pH-verschillen beïnvloed.

Produktie van PG in media met gevriesdroogd hout was veel hoger dan in die met ethanol-geëxtraheerd hout. Er was geen verschil in PG- en Cx-produktie in media met gevriesdroogd hout van resistente iepen of van vatbare iepen (Tabel 3). Geen correlatie kon worden gevonden tussen PG- en Cx-produktie en agressiviteit van stammen van *O. ulmi* (Tabel 4).

De stam TX51, die in symptoomexpressie tussen de agressieve en niet-agressieve stammen in staat (Fig. 1) maakte geen of een te verwaarlozen hoeveelheid PG. De afname in PG-activiteit tijdens de incubatieperiode, waarbij de pH omhoog ging, was niet een gevolg van vorming van PG-remmers (Tabel 5).

Acknowledgments

Thanks are due to Miss J. I. Liem for the technical assistance and to Mr H. J. Miller, Plant Protection Service (PD), Wageningen for correction of the English text.

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