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REACTIONS OF ELM WOOD TO ATTACKS OF OPHIOSTOMA ULMI (BUISM.) NANNF.

BY

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INTRODUCTION

Living plants often show the marks of developmental processes, which took place in earlier stages of life. To the observer, who knows how to interpret them, the scars on a branch of the horse-chestnut or the placing of scales and buds in the bulb of a snowdrop, reveal clearly what has happened in the past. It will always be possible to predict what may happen in future! This kind of observation turns a static picture into a dynamic one. Plant diseases may also be seen as dynamic processes, one developing out of the other. Situations are ever changing by actions of the pathogen and reactions of the host, which are interwoven. The formerly static conception of one spectacular state has nowadays given way to a more dynamic one.

History, however, is not always easy to read and reconstruction of earlier processes is often impossible without aid of experiments. This is the case with vessels, blocked by gum and tyloses, a picture well known in wood anatomy. It may be seen after invasion of parasites, such as *Endoconidiophora fagacearum* Bretz in oaks (STRUCKMEYER c.s. (1954)) or *Ophiostoma ulmi* (Buism.) Nannf. in elms, which fungus causes the Dutch elm disease. Wilting of the elm leaves correlates with a discolouration of the outer xylem, which can be seen macroscopically by peeling off of the bark of a diseased branch. Dark lines indicate the places where vessels contain a brown granular substance and where also tyloses may occur. Adjacent living cells may be filled with a dark brown substance, all cell walls may be discoloured. This static picture does not reveal history: it is impossible to reconstruct in which order of succession the deposits and the tyloses entered into the vessels.

It is a known fact, that spore suspensions of the fungus, injected into the outer xylem of an elm branch in late spring or early summer, are taken up quickly. The spores are transported over great distances in a short time (BUISMAN, 1935).

According to MAY (1934), the transport rate is about 20 cm a day. BANFIELD (1941) found even higher rates, using other methods. The velocity of transport seems to depend greatly on the season, the weather conditions and the spore loads (KUNTZ c.s., 1952).

EXPERIMENTS

In our experiments[1] the fungus could be isolated from a point 50 cm above the place of inoculation after 2 days, at a distance of 75 cm after 4 days (Table I). Somewhat behind the uppermost places, where the

TABLE I

Elm hybrid no 263 inoculated with a spore suspension on June 28.

A	B	C	D
2	20	75	0/7
		50	1/8
		25	2/8
		10	4/8
4	78	100	0/8
		75	6/8
		50	6/8
		25	4/7

A: Number of days after inoculation.
B: Length in cm of the discolouration above the place of inoculation.
C: Length in cm above the place of inoculation from where isolation was tried.
D: Nominator: number of sections, taken at the same height from which *Ophiostoma ulmi* was isolated.
Denominator: number of sections tried.

fungus could be isolated, the discolouration of the xylem was clearly visible. The changes in the darkened tissue were studied microscopically. To this purpose two hybrids were used with about the same degree of resistance against *Ophiostoma*: no 214, a hybrid between *Ulmus pinnato-ramosa* and *U. hollandica hoersholmensis* and no 263, a crossing of *Ulmus glabra* no 49 and *U. carpinifolia* no 28. Comparable branches, injected with spore suspensions at the same date, were cut at different times after inoculation. Tangential sections were cut at different distances from the point of inoculation. From sections cut from one place, 10 big vessels were chosen at random, together nearly 8 mm in length. The following parts of the vessel walls were measured: 1. the lengths of the discoloured places; 2. the lengths of the parts covered with gum; 3. the lengths of the parts covered with tyloses and gum or with tyloses only and 4. the lengths over which the lumina were blocked. Fig. 1 shows, that in no 214, at a height of 7–12 cm above the point of inoculation, the discolouring of the walls of the vessels and the tracheids were the first phenomena that manifested themselves clearly. In the living cells a dark content began to show. Nearly at the same time exudation of druplets of gum occurred. After

[1] Some of which were performed by Miss A. P. Vink, biol. cand.

piercing through the pit cavities smaller and larger globules accumulated in the vessels. The first colourless tyloses appeared about 5 or 6 days later. They always developed out of living cells bordering non-discoloured parts of the vessels. If gum, originating from discoloured cells, was already present, the tyloses pushed these deposits aside.

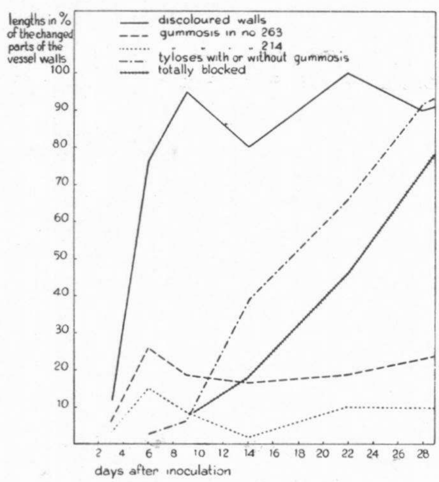

Fig. 1. Changes in the walls of the vessels taking place in 4 weeks.

They filled the lumina of the vessels at a high percentage. In a tangential direction from heavily attacked vessels, others were found with only tyloses, which developed out of healthy looking living cells. When these processes had already started at the base of a branch near the place of injection, the vessels at the top might just be reached by spores. The same succession occurred here at a somewhat later date. Till now mycelium has seldom been seen in the vessels of diseased elms (VAN VLOTEN, 1942). In one of the branches used in our experiments, hyphae were also seen, growing outwards into the vessels in perpendicular direction, bearing conidia near the top. Probably this mycelium had developed out of spores, which after transport had halted against the protrudings of the vessel walls.

ZENTMYER (1942) and others consider the toxin formed by the spores to be the causal factor of the anomalities. To compare the effect of the sucking in of a spore suspension with that of toxin, the fungus was grown in a nutrient solution[2] during 5 days. Following centrifuging the liquid was filtered until no more spores were present. The method of inoculation was that used by BANFIELD (1941): plastic cones were affixed to branches of *Ulmus hollandica belgica* and filled with the suspension or with the filtrate. Chisel cuts were made below the level of the fluid into the outer xylem.

It appeared, that the effect of the toxin was comparable to that of

[2] KH_2PO_4 1.5 g; $MgSO_4 . 7H_2O$ 1 g; glucose 25 g; asparagin 2 g; yeast extract 2 g; aqua dest. 1000 g.

TABLE II

Ulmus hollandica-belgica inoculated with spore suspension and with toxin on June 11.

Number of days after inoculation	Spore suspension [1])	Toxin [1])
2	8/165	30/135
4	40/145	42/145
8	60/140	50/195
26	140/140	130/130

[1]) Nominator: length of the discolouration of the branches above the point of inoculation in cm. Denominator: total length of the branches.

the suspension, save for the first 2 days (Table II). After that period the toxin had produced a discolouration of 30 cm and the spore suspension one of only 8 cm. After 4 days the xylem had darkened in both cases over resp. 42 and 40 cm. This result can be explained by the formation of toxin by the spores, after their entrance into the vessels, which process probably took a couple of days. Microscopically the same phenomena were seen in the vessels as well after toxin had entered, as after the transport of spores. Injections with nutrient solution and with water also caused slight reactions in the vessels.

Discussion

Chattaway (1949) puts forward, that both gums and tyloses are a manifestation of the reaction of living ray cells to a stimulus which causes increased activity. In which form this activity is shown, depends on the size of the aperture of the pits between the vessels and the living cells of the medullary rays. If this size is large, the pit membrane is forced into the vessels by the expanding cells. If on the contrary these pits are small, expansion is impossible. The reaction is then shown by secretion of substances by the cell into the vessel cavity. At a pit aperture of 8–10μ both reactions may be seen and gum druplets as well as tyloses may occur in blocked vessels. In the Dutch elm the size of the aperture of the pits between the big vessels and the adjacent cells of the medullary rays is about 6–8μ. Smaller and larger pits are scattered over the walls at random. According to Chattaway gum globules would enter the vessels through the smaller and tyloses through the larger apertures. This is not the case. There are parts of the walls, where only gum appears through all pit membranes and other parts, with only tyloses, whatever the size of the pits.

The common incitant for both types of reaction, must here be sought in the toxin. The spores, which produce the toxin, are without doubt unevenly distributed in the vessels, halting at places where protrudings occur, or where vessels are divaricating. At places where branches are forked, dark discolouration of the wood, indicating the presence of gummosis, are often more pronounced than elsewhere. Clusters of spores may occur at those places, producing toxin locally at a high concentration. Adjacent living cells exposed to such a high concentration may be inclined to gummosis. Probably the mycelium that was observed, occurred at such a place. Other cells, receiving toxin at a lower rate, may be stimulated to enlarge.

STRUCKMEYER c.s. (1954), considering the lignification of the walls of the tyloses in oaks a normal process, suggests, 'that a modification of the normal host cell metabolism has taken place, rather than that some drastic toxic action has occurred'. But the influence of toxin at low concentrations does not need to be 'drastic'! From a centre with a high concentration, toxin will be distributed into a tangential direction to neighbouring vessels, where the concentration will become low enough to cause formation of tyloses.

This is in accordance with the observation that centres of heavy attack are surrounded by 'courts, where the parasitic influence is less severe' (BROEKHUIZEN, 1929). It is also possible, that it is not the toxin but other compounds, originating from the damaged cells in the centre, that induce tyloses at some distance. In that case the formation of tyloses may be compared with the enlargement of cells under the superficial 'blocked layer' in other damaged tissues.

Not all hybrids react in the same way on injections with a spore suspension. After inoculation, no 263 showed a higher degree of discolouration than no 214, which is due to a greater amount of gum deposited in the vessels (Fig. 1). Spores may be kept imprisoned in the sticky masses. It was possible to detect their presence by isolation of the fungus, even after a long period.

It is suggested by WARDLAW (1935), that in case of the Panama disease of bananas, caused by *Fusarium oxysporum var. cubense* (E. Sm.) Wr. blocked vessels may provide means by which fungal penetration might be inhibited. In that case the rate between the rapidity of the advancing hyphae and the rapidity with which gum and tyloses are formed, may determine the susceptibility of a tree. Till now, however, no such a hypersensitive elm hybrid is found, for the advancement of the spores is much greater than the rate of the formation of gum. It will be important to compare the behaviour of the living cells adjacent to the vessels in a more resistant hybrid with that of a less resistant one. This study may perhaps give some insight into factors concerned with resistance.

SUMMARY

The reactions of the xylem of *Ulmus* hybrids were studied after injecting branches with a spore suspension of *Ophiostoma ulmi* (Buism.) Nannf. Discolouration of the walls of the vessels and formation of gum droplets in the pit cavities, were followed by development of tyloses. The expanding living cells always pierced through those pits where gummosis was absent. The toxin produced by the spores will be the causal agent of these anomalities. At places where many spores adhere to the walls of the vessels, gummosis may occur. At other places, where toxin is less concentrated, tyloses may appear.

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