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VEGETATIVE PROPAGATION OF ELMS BY MEANS OF SHOOTS CUT FROM CALLUSED ROOTS

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Abstract

A method is described for the propagation of elms from cuttings, raised from root-callus in the glasshouse. The technique applied is a modification of that described by Doran and McKenzie (1949).

Shoots of adequate size, developed from the callus, were excised and planted. In early summer, they could be hardened in a cold frame.

Callus cuttings were raised in different months of the year. The best results were obtained with root segments cut during the dormant period of the elms. Shoots excised in early spring rooted readily under improving light conditions.

From the results of investigations since 1959 it is apparent that seedlings, as well as clones, can be successfully propagated in this way.

Different clones of elm have shown differences in ease of propagation. The Dutch elm, *Ulmus hollandica* "Belgica", produced less callus and shoots on root segments than other clones. Clones No. 1 and No. 148 were good producers of cuttings: from one elm of 2 to 4 years old about 80 genetically equal individuals on their own roots could be obtained, representing homogeneous material.

The callus cuttings can be used in the study of parasitism of *Ceratocystis ulmi*, e.g. for testing the virulence of the fungus. They may also be used for selection purposes. From a seedling apparently resistant to the Dutch elm disease, a clone on own roots can be grown for further testing and thus any influence of the stock on the scion is obviated.

Comparison of young callus cuttings and stem cuttings of the same age made it clear that the former represent the juvenile and the latter the adult stage of the tree. Both are sensitive to the fungus from the second year of growth on. The hypothesis of insensitivity of elms during the juvenile stage, the so-called youth-resistance, seems to be improbable.

Introduction

In the course of studies on Dutch elm disease caused by *Ceratocystis ulmi* (Buisman) C. Moreau it became apparent, that there was a need for a rapid and efficient method of propagating elms vegetatively. There were two main reasons for this. Firstly, it was desired to compare the virulence of different isolates of the pathogen. The wide genetic variability of elm seedlings in respect of susceptibility to the disease (even when a single isolate of the fungus was used) made them quite unsuitable for this purpose. Secondly, lack of visible disease symptoms on a particular tree, even after repeated inoculation, was not sufficient proof of its resistance. It was therefore frequently necessary either to

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test the resistance of seedlings of the tree or to inoculate a number of shoots from the tree, that had been grafted onto rootstocks, usually of a susceptible commercial variety. Such grafted scions often showed great variability in growth, however, and there was also the possibility of a stock-scion effect. It would obviously be advantageous, therefore, to be able to inoculate a large number of uniform replicate trees, all of the same genetic constitution and all growing on their own roots.

For a number of years experiments have been performed in different countries in order to obtain stem cuttings of elms on their own roots by vegetative propagation (DORAN, 1957; OZOLIN, 1958; MATTHEWS & JOBLING, 1960; BROEKHUIZEN, 1961; OUELLET, 1962). In our experiments, however, it became evident that not all elm species and hybrids are suitable for propagation from stem cuttings. Moreover a mist installation is necessary for growing such cuttings. As early as 1949 DORAN and McKENZIE pointed out the possibility of propagating the resistant and highly esteemed "Christine Buisman"-elm by root cuttings. Roots were divided in portions, which were planted vertically in a rooting medium. A ring of shoots developed from callus on the cut surface of the roots.

The shoot which developed best was kept and the others were cut away. When treated in this way the rooting of the "Christine Buisman"-elm was about 80 % according to BRETZ and SWINGLE (1950). When this method was tried in the glasshouse of the Phytopathologisch Laboratorium "Willie Commelin Scholten", it was found that the shoots which grew from the callus could be removed and rooted. This fact indicated, therefore, a method whereby several plants, instead of a single one, could be grown from each segment of root.

Following preliminary experiments performed in 1959 in a heated glasshouse, cuttings are now made from callused roots during a part of the year. They show luxuriant growth, if well treated, and their uniformity is striking. In this paper they will be referred to as "*callus cuttings*".

METHODS

Earthenware seed trays 25 × 25 cm in size are used. A medium consisting of a mixture of sand and peat in the ratio of 1:3 is most satisfactory. The roots to be used are from 3 to 15 mm in diameter. They are usually cut just before planting into segments about 7 cm long, which are inserted vertically into the rooting medium with the proximal end uppermost and protruding at least $^1/_4$ cm above the soil. In this way 40 to 50 root segments can be planted in one tray. Immediately after planting the trays are covered with thick sheets of polythene suspended on sticks at a height of about 20 cm above the cuttings. After 2 weeks a green ring of callus tissue protrudes from the cambial region at the cut surface and lateral roots start developing (Plate 1A). The callus tissue begins to differentiate: meristems are formed, giving rise to adventitious buds which may develop into shoots (Plates 1B and 2A). Eight to ten weeks after insertion of the roots some of the shoots may bear six to eight leaves (Plate 1C). At that time the stems may become woody with the beginning of cork formation. The ones at this stage are carefully cut off at the base and dipped into a root-inducing substance "Rhizopon" for a length of about 1/4 cm in order to promote root development. The powder is shaken off and the shoots are planted into preformed holes in the planting medium. This has the same consistency as the medium used for the root segments. Ten shoots are planted in a 5-inch pot, each one of which is closed with a plastic bag to maintain a high humidity. The pots are placed on a bench in the heated glasshouse, immersed in moist peat.

After the removal of the first batch of suitable shoots, smaller ones still emerging from the callus ring are able to grow to an adequate size for cutting. In this way it is possible to cut shoots repeatedly from one segment of root. In the experiments described in this paper only two batches of shoots were cut. Adding some manure to the rooting medium, however, made it possible to cut four lots of shoots from one root. The shoots from the first and the second batch rooted equally well and rooting usually started 10 to 12 days after cutting and planting. The rooted cuttings can be potted in a good garden soil.

Shoots inserted in February are far enough developed about June to be put into cold frames, where they are hardened off. In the spring of next year they can be planted out in the field and later they can be tested for resistance or used for testing the virulence of *Ceratocystis*-strains.

Results

Of the many experiments performed since 1959, only those are discussed here of which the results could be expressed numerically, i.e. as the percentage of root segments that formed callus, the number of shoots cut in two successive batches and the number of well rooted and developed plants that were ultimately obtained.

Experiments with roots of seedlings

On 9 February between 42 and 49 root segments were cut from each of four 3-year-old seedlings, which had survived repeated testing for resistance. The roots were planted immediately after cutting.

Table 1a shows that most of the roots formed callus, from which a number of well grown shoots developed. From each segment of root an average of about 3 shoots could be cut in the two batches. From 82 % to 91 % of the shoots developed into healthy plants after they had rooted. From each seedling 88 to 125 callus cuttings were obtained. During the summermonths the plants were large enough to be hardened in cold frames and growth continued without shedding of leaves. In spring of the next year the plants could be transplanted into the field.

Tests were also made of the effect of planting root segments at various times of year. During the period from April until August cambial activity in the roots caused a loosening of their cortex and,

TABLE 1.
Callus formation on root segments and rooting of callus cuttings.

No. of seedling	number of root segments		number of shoots		number of plants grown	
	inserted	callused (in %)	inserted	rooted	in % of the number of shoots	in % of the number of root segm.
		a. roots inserted in February				
TA II	45	42 (93)	108	88	82	196
TA III	43	40 (93)	136	119	88	277
TA I	45	43 (96)	139	125	90	278
TA IV	42	38 (90)	117	106	91	252
		b. roots inserted in August				
52/15	42	31 (74)	187	60	32	143
66/28	44	43 (98)	187	115	61	261
67/18	49	42 (86)	229	119	52	243
44/15	42	37 (88)	101	83	82	198

when they were cut into portions, the cambium was easily damaged and callus development was only poor. It also appeared that the root segments formed only very few lateral roots or no roots at all and there was thus an insufficient supply of nutrients for the development of the young shoots. The few shoots, that still developed on the inserted root segments, died prematurely.

Callusing and shoot production was satisfactory when the root segments were inserted during the period from the end of August until November. Table 1b shows the result of an experiment with root segments inserted on August 22. They were cut from four 8- to 9-year-old seedlings. The percentage of root portions that callused was somewhat lower than after planting in February. The number of shoots obtained was rather high. They were cut off and were grown under artificial lighting during the winter. The percentage of the shoots that rooted was low and the further development of the plants was not satisfactory. The plants remained in a weak condition. After they were put in the cold frame, most of the leaves were shed and the plants became dormant until the next spring. This behaviour may be due to a shortage of light, notwithstanding the artificial lighting, during the period of development of the shoots. At the time of transport to the cold frame they may not have been able to stand the lowering of temperature, resulting in dormancy.

On the other hand shoots derived from roots inserted in February developed well. During the following months light conditions became more and more favourable. These shoots showed excellent growth which was not inhibited by the sudden change in conditions when they were removed to the cold frame. The same effect was obtained with roots excised in October but kept under a layer of about 5 cm soil outdoors during the following 4 months. After these root portions were inserted in February, callus was produced and the shoots developed well. When the root segments of the same batch were

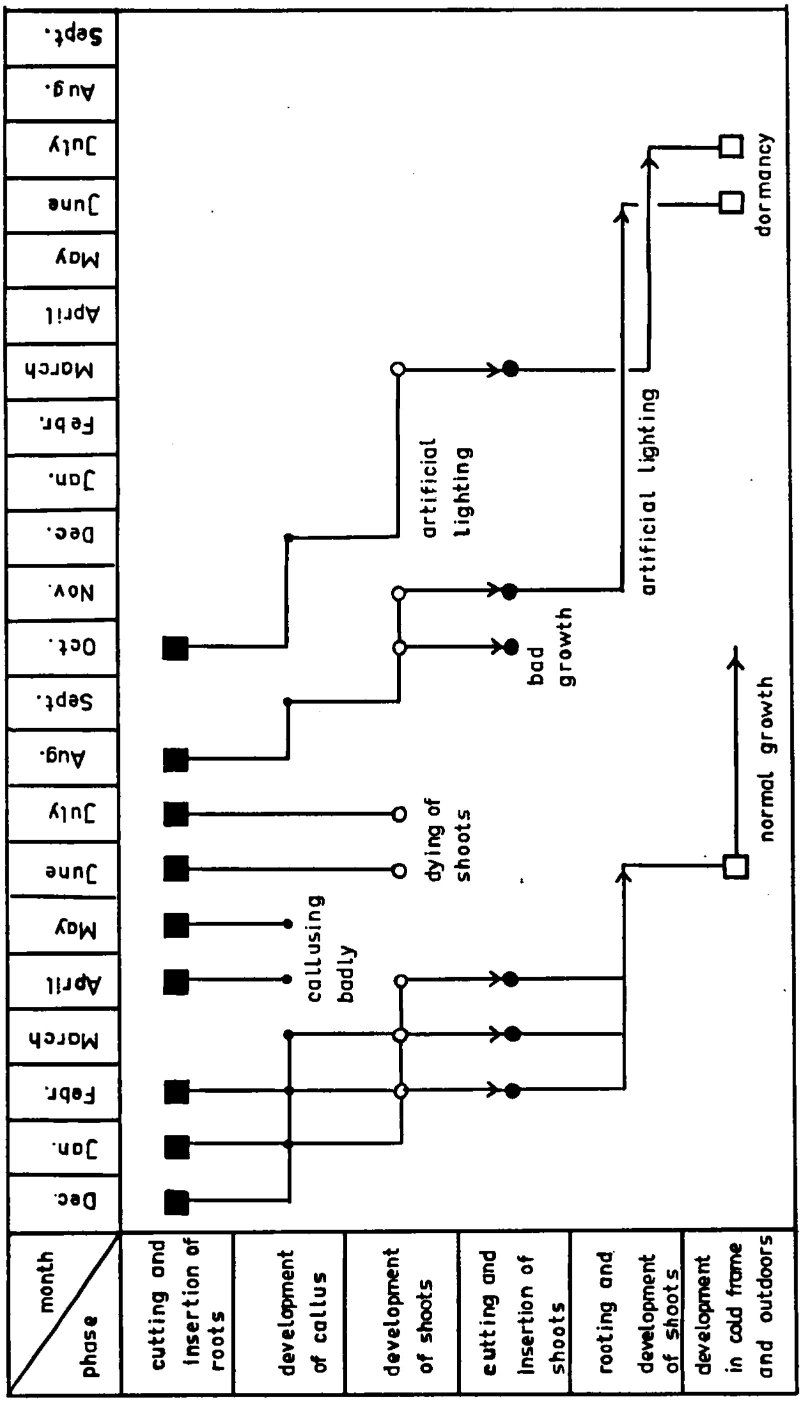

Fig. 1. Scheme of the vegetative propagation of elms by means of root callus cuttings in different months.

inserted immediately after cutting in October, however, the shoots they formed did not root well and became dormant later on.

Fig. 1 represents a scheme of the developmental stages of vegetative propagation in different months. It may be concluded that the best time for inserting root segments will be from December to and including February, the dormant season. The time of the year during which shoots are excised is also important. Shoots inserted in February to and including April will root readily and their growth is not inhibited when they are transferred into the cold frame.

Experiments with roots of clones

The method of vegetative propagation described above was also applied to eight different clones, raised by the Forestry Experimental Station "De Dorschkamp" by layering or by means of stem cuttings. The age of the trees varied between two and four years. Root material of all the clones was gathered simultaneously in August. This month had appeared to be unfavourable for obtaining callus cuttings as they would enter into dormancy after being placed in the cold frame (Fig. 1). The purpose of this experiment, however, was to assess the number of cuttings which might be grown from the inserted root portions, irrespective of their ultimate reactions to the process of hardening.

The numbers of root segments that could be obtained from each of the eight clones were unequal, as the extent of the root systems differed widely. Moreover it had become evident from several preliminary experiments, that obtaining callus cuttings of the Dutch elm (*Ulmus hollandica* "Belgica") was difficult. For that reason twice as much root material was gathered from two trees of *Ulmus hollandica* "Belgica" as from the other clones. Callus cuttings were raised in the glasshouse.

A distinct difference in behaviour between the clones became evident (Table 2). The numbers 1 and 148 showed the best results. The number of shoots that could be excised in two batches was almost twice the number of root segments inserted and 90 % of the shoots

TABLE 2

Results with callus cuttings of eight elm clones. Roots inserted in August.

clone	number of root segments		number of callus shoots		number of plants grown	
	inserted	callused (in %)	inserted	rooted	in % of the number of shoots	in % of the number of root segm.
No. 1	45	33 (73)	90	81	90	180
No. 148	50	31 (62)	91	83	91	166
No. 248	40	12 (30)	10	8	80	20
No. 274	40	11 (28)	21	8	38	20
Americana No. 3	50	27 (54)	50	44	88	88
Ch. Buisman	35	25 (71)	42	32	76	91
Bea Schwarz	45	19 (42)	22	21	95	47
U.h. "Belgica"	108	16 (15)	11	4	36	4

rooted. From each mother-tree about 80 individuals could be raised—a very satisfactory result. In contrast with these clones, *Ulmus hollandica* "Belgica" resisted efforts to propagate it by means of callus cuttings. Callus formation as well as rooting of the shoots was unsatisfactory. Only 15 % of the roots callused and 36 % of the planted shoots became rooted.

The results of other experiments with the Dutch elm differed widely and it seems likely that the presence of roots, suitable for vegetative propagation, depends on the origin of the trees of this clone, which has been in cultivation for centuries. Results obtained with elms from a certain nursery were always better than those with elms grown in another nursery. It is possible that by selecting trees, which formed only few root suckers, the ability to develop adventitious shoots from roots has also been suppressed. Seedlings of the Dutch elm, derived from freely pollinated seed, could usually be propagated readily by means of callus cuttings.

TABLE 3

Results with callus cuttings of Ulmus hollandica "Belgica". Roots inserted in different months of the year.

Origin	month of insertion	number of root segments		number of callus shoots		number of plants grown	
		inserted	callused (in %)	inserted	rooted	in % of the number of shoots	in % of the number of root segm.
Dutch-elm (layers, 2 to 4 years old)	February	51	27 (53)	64	47	73	92
	June	49	8 (16)	27	2	8	4
	August	108	16 (15)	11	4	36	4
	October	92	33 (36)	62	39	63	43
	December	307	144 (47)	180	116	64	38

To obtain data concerning the best season for inserting roots of *Ulmus hollandica* "Belgica" experiments were carried out in different months of the year (Table 3). In order to avoid the influence of differences in origin on the final results, roots of the same origin were used in all experiments.

The results obtained in different months varied widely. They were satisfactory after inserting root segments in February and growing shoots in spring. Growing shoots during the summermonths up to September failed, however, for the most part. Results improved after inserting roots from October onwards, being best from material collected and inserted during the dormant period from December to and including February. The behaviour of the Dutch elm, therefore, also fits into the scheme of Fig. 1.

CONDITIONS INFLUENCING THE RESULTS OF PROPAGATION BY MEANS OF CALLUS CUTTINGS

In raising callus cuttings two independent rooting processes have to be considered: the rooting of the inserted root segments, which determines the rate of development of callus and shoots on the cut surface, and the rooting of the excised shoots, leading to the development of young elms on their own roots. If root development fails the young shoots will die sooner or later.

Several factors influence the results of this method of vegetative propagation. It appeared that a temperature of 21° to 25° C was optimal and that a relative humidity of 80 % of 85 % prevented the cut surface of the roots from drying, allowing callus formation. At a higher relative humidity fungal growth on the cut surface was followed by rotting of the callus ring.

From a number of experiments it was found that a medium of peat and sand in a ratio 3:1 was most successful. This was in agreement with the experience of BROEKHUIZEN (1961), who recommends this medium for stem cuttings of elms. If the quantity of sand added to the medium was too high, so-called "callus nodules" instead of roots were formed at the base of the excised shoots.

Next to the environmental conditions it is the internal condition of the material that counts. The following factors are of importance:

a. The age of the mother-tree, from which the roots were cut, influenced the rooting of the inserted root segments. Results achieved with material derived from 2 to 4 years old trees were better than those obtained with material cut from an adult tree at the stage of flowering.

b. The diameter of the root segments was of less importance for callus- and shoot development, unless it was greater than 15 mm. Those with a diameter larger than 15 mm rooted poorly or not at all and the shoots developing from the callus died off.

c. The influence of the site of the root portion in relation to the base of the root system of the mother-tree was not determined. Shoot development took only place at the proximal end of each root segment.

d. Most important is the stage of development of the shoots at the time of cutting. Their ability to root well and therefore the final results of the propagation depend on the choice of the shoots. It is impossible to prescribe a definite stage suitable for cutting, as woodiness of the stem-base of the shoots is an important character indicating ripeness to cut. The presence of woodiness depends on the density of the ring of shoots on the cut surface of the root segment. If there are many sprouts, development of individual shoots occurs only slowly. If a shoot is sufficiently developed and shows elongation it can be cut and will usually root within 2 weeks.

e. Growth substances are a considerable help in promoting root development of the callus cuttings, though not all commercial preparations were satisfactory. In some cases the shoots could be transplanted as soon as 3 weeks after insertion.

f. Fungal attack occurred specially at high temperatures and high relative humidity of the air in the glasshouse. A treatment of the affected cut surfaces with TMTD (thiram; tetramethylthiuramdisulfide) inhibited fungal development; preventive treatments, however, were not effective.

g. Conditions have to be chosen in such a way, that going over into dormancy is prevented after leaving the glasshouse. Light and temperature are determining factors. If either the light is too weak or the temperature is too low, the callus cuttings may become dormant. This may happen at all developmental stages. Even shoots still present on the cut surface of the root segments may stop growing under unfavourable conditions. Shoots cut from October until the end of the year are likely to become dormant. Those cut from February to April show good growth in the glasshouse at high temperatures. Even they have to be hardened carefully before they could be exposed to the open in June. If the transport from the glasshouse to the cold frame results in a sudden decrease in temperature the possibility of entering dormancy is great. If the callus cuttings keep on growing and go over into dormant state normally in autumn, it will be possible to produce well developed plants about $1^1/_2$ year old in the next summer.

Juvenile character of the callus cuttings

The first developmental stages during which a plant differs from the adult plant in morphological and physiological respects is called the "juvenile stage" (Kobel, 1954; Blair, McArthur and Nelson, 1956). Juvenile characters can be found in the morphology of the leaves or in the branching and also in physiological behaviour: a juvenile plant is still in the vegetative stage, being unable to flower. The ability to form adventitious roots, however, may be well developed. The characters of a juvenile plant gradually pass over into those of the adult stage. Each juvenile character, however, has its own period during which it is maintained. Therefore a plant may be considered as juvenile in respect of one character but adult in respect of another. The limit between the two stages can never be defined exactly. The elm shows its juvenile character longest in relation to flowering.

It is still unknown when an elm starts to be susceptible to Dutch elm disease. Attempts have often been made to select young elm seedlings resistant to *Ceratocystis ulmi* by inoculating them with a spore suspension of the fungus. These experiments failed as none or only few of the plants reacted with visible disease symptoms to the presence of spores. A high percentage of the seedlings became diseased when testing was repeated at a later stage. It is assumed that elms are resistant in the juvenile stage (Heybroek, 1957). On the other hand it may be true that the way the plants were treated led to an apparent resistance, which, in fact, was no more than insensitivity. If this is indeed the case, selection of seedlings resistant to the disease might be possible by raising them under such conditions that they are able to show external symptoms after inoculation with spores of the fungus.

For testing young elm plants for disease resistance it was necessary to compare the susceptibility of two groups of plants of the same age and belonging to the same clone: one group in the juvenile and other in the adult stage. This possibility exists with the elm as stem cuttings obtained from an adult tree have to be considered as rooted parts in

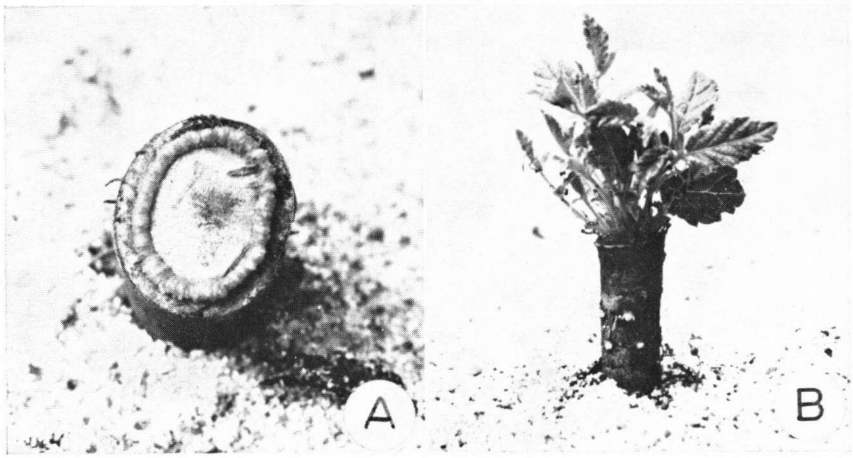

Plate 1. A. Callus development two weeks after insertion of root segment. B. Callus shoots on the cut-surface of the root of a Dutch elm (*Ulmus hollandica* "Belgica").

C. Root segment of "Ch. Buisman"-elm showing shoots in a stage of development suitable to be excised.

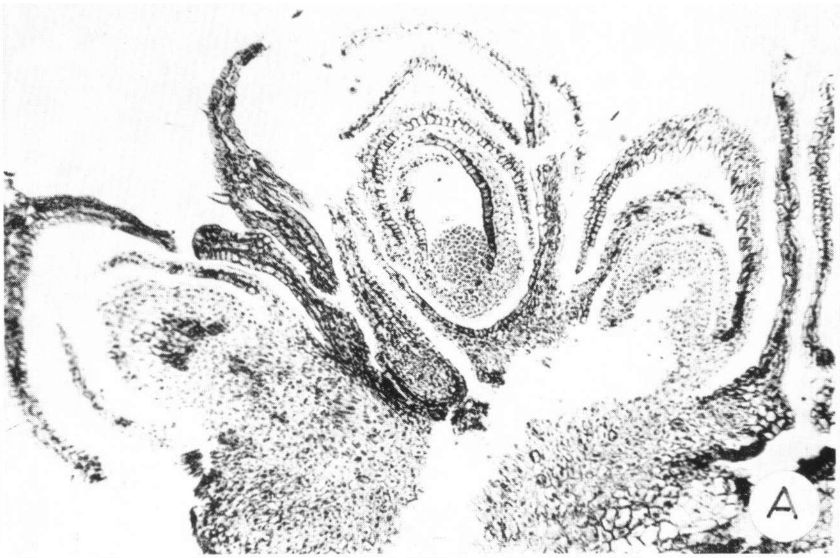

Plate 2. A. Callus buds, neoplasms from the cambium zone of a root segment.

B. "Bea Schwarz"-elm. Right: callus cutting in juvenile stage. Left: stem cutting in adult stage.

that stage. They maintain the adult character. From the same tree callus cuttings out of adventitious buds, derived from cambium zone of the roots, can be grown. These neoplasms are in a juvenile stage of development. It is thus possible to grow two groups of plants of the same age, originating from the same tree: stem cuttings in the adult and callus cuttings in the juvenile stage. They are equal in genetic respect.

Stem- and callus cuttings of the Dutch elm, the 'Bea Schwarz'-elm and of clone 248 were simultaneously raised. It was observed that the callus cuttings of the three clones at the age of one to two years differed morphologically from the respective stem cuttings. The differences shown by the two groups of the "Bea Schwarz"-elm were most striking. In comparison with the stem cuttings, the leaves of the callus cuttings were more pointed at the apex and their margins more sharply dentated; the leaf surface was hairy and rough. The lateral twigs of the callus cuttings formed right angles to the stem or the angles were even obtuse; the laterals of the stem cuttings formed acute angles (Plate 2B). Anatomical differences were also perceived: the xylem of the callus cuttings showed far less vessels than that of the stem cuttings. The xylem of the former also contained less parenchyma and only a small number of medullary rays. The summerwood of the stem cuttings showed isolated, irregularly formed groups of small vessels, surrounded by other elements. These so-called "îlots de Morus" (HOULBERT, 1899) also occur in the wood of old elms, where these "isles" are present in tangential bands.

From the results of experiments it could be concluded that the callus cuttings, occurring in a juvenile stage, reacted with visible symptoms of the Dutch elm disease after they were inoculated with a spore suspension of *Ceratocystis ulmi*. The hypothesis of youth-resistance seems to be improbable. Also from the experiments of ARISUMI and HIGGINS (1961) with young seedlings it may be concluded that youth-resistance of elms is unlikely.

Another way, in which juvenile plants on own roots could be obtained, was by cutting shoots from callus developed on the surface of a stem cut at the base.

A. Kramer, superintendent of our glasshouse, has succeeded in growing cuttings from callus developed on a stem of the well-known *Ulmus hollandica* "Vegeta", a clone much planted in practice with a relative high degree of resistance against the Dutch elm disease. The stem was cut just above the junction with the stock and callus cuttings could be raised, which showed morphological juvenile characters. Further vegetative propagation from this material may be possible within 3 or 4 years. In this way "Vegeta"-elms on own roots will be available for use in the field trials and for practice. This way of raising cuttings suggests a new possibility of propagating clones on their own roots.

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