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Physiological changes in M 26 and M 106 softwood apple (*Malus* sp. Mill.) cuttings during their rooting

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Abstract

The levels of rooting cofactors, monohydroxyphenols, o-dihydroxyphenols, total phenols and protein, as well as the activity of peroxidase and polyphenol oxydase were compared in the leaves and stems of untreated vs IBA-treated cuttings. The cuttings of M 26 and M 106 clone were analysed on days: 0, 16 and 24 of rooting. Marked differences were found between the clones and treatments compared in levels of the substances analysed but these were not correlated with the rooting ability of cuttings. Only low monophenols' content in the IBA-treated M 106 cutting stems was correlated with the best rooting obtained.

INTRODUCTION

The physiological interrelations between auxins, phenolic substances and IAA-oxidizing system (s) are still not fully understood with respect to adventitious root formation on stem cuttings (van der Mast, 1970a, b; Psenak et al., 1970; Basu, 1971; Sirois and Miller, 1972).

In spite of this many phenolic compounds have been identified as rooting cofactors (Lanphear and Meahl, 1963; Hess, 1964, 1968; Lipecki et al., 1975; Stoltz and Hess, 1966b; Fadl and Hartmann, 1967) and many phenolic compounds administered to the cuttings promoted their rooting, acting as auxin synergists (Basu, 1970; T. Bojarczuk and Jankiewicz, 1975; K. Bojarczuk and Jankiewicz, 1975; Poapst and Durkee, 1967; Jankiewicz et al., 1973; Piątkowski et al., 1973; Sadhu et al., 1978).

The effect of IBA on the rooting of apple softwood cuttings was promotive in experiments of Górecki (1979) as is well known in the case of many species.

The aim of this work was to compare physiological changes in rooting cofactors, phenolic compounds, enzymes' activities and protein con-

tent in IBA-treated cuttings of M 26 (which rooted poorly) and M 106 (which rooted well) during the first weeks of rooting.

MATERIAL AND METHODS

Experiments were done in the Dept of Plant Physiology, Agricultural Academy in Poznań.

1. PLANT MATERIAL PREPARATION AND TREATMENT

Softwood cuttings of rather difficult-to-root M 26 and easier-to-root M 106 clone were gathered on July 6th (expt 1) and August 24th (expt 2), 1972. They were rooted for 49 days as described in details previously (Górecki, 1979). The samples of 16 cuttings were tested on the day 'zero' of setting the experiments and then after 16 and 24 days of rooting. The cuttings were divided into leaves and stems which were analyzed separately. The cuttings compared were: controls dipped in talcum-captan (4:6 wt/w) powdered preparation and those treated in addition with 5000 ppm of IBA in the above preparation.

The leaves and stems were cut into small pieces and preserved as described below, for further analysis. The protein content, phenolic compounds, rooting cofactors, polyphenoloxidase and peroxidase were determined.

2. PLANT MATERIAL CONSERVATION AND EXTRACTION

For determination of polyphenols fresh tissue samples (4.0 g) were conserved as follows. The tissue cut into small slices was placed into 50 ml flasks with 30 ml of boiling ethanol, taking care that all material was covered. Then the tissue was stored at −15°C.

For rooting cofactors 2.0 g samples of fresh material were put into flasks. Boiling ethanol (30.0 ml) was poured into the flasks and boiling maintained for 1 min. Then the samples were stored at −15°. Just before extraction the samples were boiled under a condensor for 15 min. Tissue was homogenized with fresh portion of 80% ethanol for 4 min (5 ml per 1 g of tissue). The residue was boiled again for 15 min and the extracts were combined. The protein content and enzymes were analyzed in the acetone powders (see below).

3. METHODS OF DETERMINATION

Phenolic compounds. Total phenols were determined with the method of Swain and Hillis (1959) and o-diphenols with the method described by Johnson and Schaal (1957), using chlorogenic acid

as reference. Monophenols were determined after passing of extracts through columns with aluminum oxide according to the procedure described by Krzywański (1975) and using ferulic acid as reference.

Rooting cofactors were determined using the mung bean (*Phaseolus aureus* Roxb.) bioassay, after Hess (1964). The extract equivalent to 125 mg of fresh tissue was spotted in a line onto chromatographic paper as described by Krzywański et al. (1976).

The protein and enzyme activity were determined in acetone powders. They were obtained as follows: 2 g samples of fresh material were ground for 5 min in 15 ml of cold (−15°C) acetone. The residue was washed ten times with 20 ml of cold acetone to obtain a colorless substance. Powders were dried in the air and stored in a refrigerator untill needed.

The enzymes: polyphenoloxidase (PPO), (1.10.3.1) and peroxidase (PO), (1.11.1.7) were determined in extracts obtained as described by Krzywański et al. (1976). The activity of PPO was determined according to the method given by Fehrmann and Dimond (1967) with prolonged time of the second reading. The activity of PO was determined after Bojarkin (1954).

The protein content was determined by the method of Lowry et al. (1951) using albumin (bovine plasma) as reference.

RESULTS

The results presented are based mainly on the experiment (No. 2) set up on Aug. 24th, since the differences in rooting were more pronounced at that time (Table 1).

The rooting cofactor activity in extracts from leaves and stems differ-

Table 1

The effect of 5000 ppm IBA in powdered talcum-captan preparation on the rooting of apple softwood cuttings after 49 days (%). The experiment No. 1 was set up on July 6th, and the experiment No. 2 was set up on August 24th, 1972

Clone	Treatment	Number of experiment	
		1	2
M 26	control	0.0	2.1
	IBA	6.3	14.6
M 106	control	2.1	2.1
	IBA	25.0	77.1

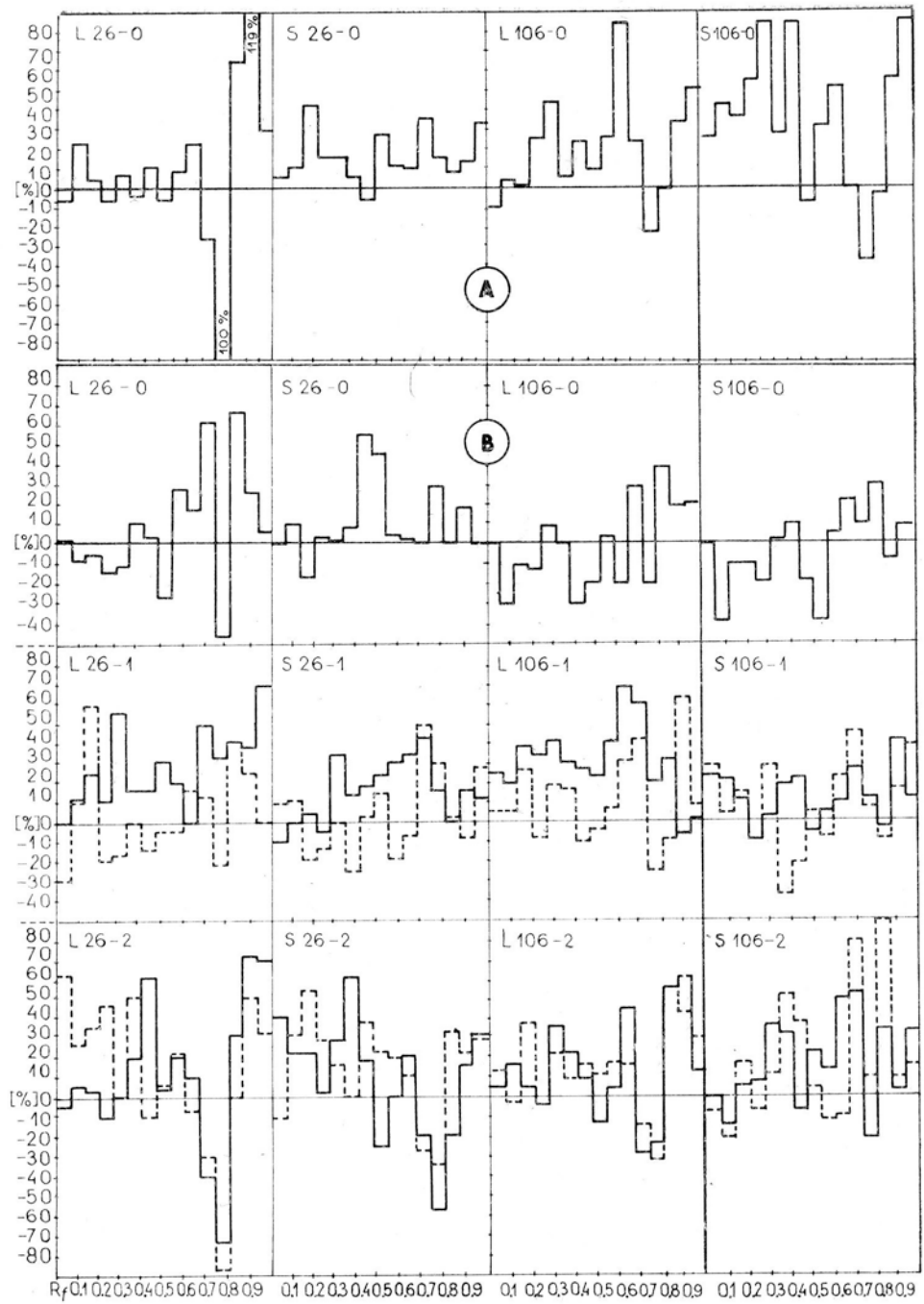

Fig. 1. Mung bean bioassay for rooting cofactors in the leaves (L) and stems (S) of softwood M 26 (26) and M 106 (106) cuttings, on day 0 (0) and after 16 (1) and 24 (2) days of rooting. Experiment 1 (started at July 6th) — A; experiment 2 (started at August 24th) — B. Per cent of stimulation (+) or inhibition (−) in relation to the control are indicated

red markedly in the two cultivars at the beginning of the experiments (Fig. 1A and 1B — "0" row). In particular, a noticeable zone of rooting inhibitors was observed with M 26 leaf extracts at R_f 0.8 in contrast to M 106 clone. Also, there were differences due to IBA application on a course of rooting (Fig. 1B). It is difficult, however to decide the direction in which IBA acted. Generally, IBA caused a decrease of rooting cofactors after 16 days of rooting and an increase after 24 days, but anew, after 24 days the strong inhibition zone was present in the extracts of M 26 leaves and not in M 106, at R_f 0.8. When the chromatograms were compared unter UV light (+NaOH) it was noted that all

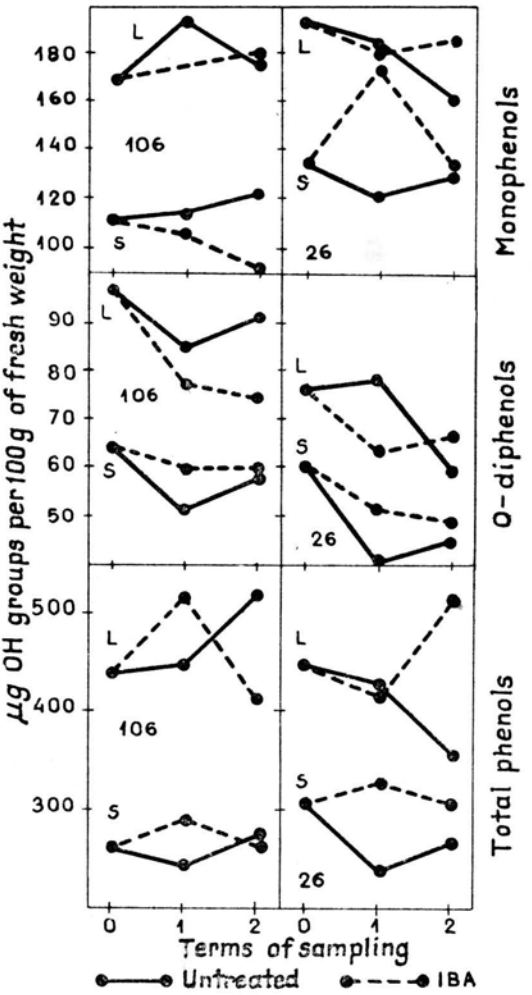

Fig. 2. The content of phenolic compounds in the leaves (L) and stems (S) of IBA-treated M 26 (26) and M 106 (106) cuttings after 0, 16 and 24 days of rooting (0, 1, 2, respectively). Experiment 2

the stripes seemed to contain the same substances but only the intensity of their bands' coloures were different. This presupposition is in part confirmed by data regarding the content of phenolic compounds in leaves and stems of cuttings, during the time of their rooting (Fig. 2).

The content of phenolic compounds was always higher in leaves than in the stems of the clones investigated, regardless of the treatment and group of phenols taken into consideration (Fig. 2). (The data from the first experiment, not included in the figure, showed generally similar tendencies). The content of o-diphenols and total phenols increased in the stems of M 26 and M 106 cuttings treated with IBA and usually decreased in their leaves. The content of monophenols decreased in the leaves of IBA-treated M 106 cuttings. It is interesting, however, that in the stems of IBA-treated M 106 cuttings the content of monophenols declined, but in the stems of M 26 markedly increased after 16 days of rooting and returned to the initial level after 24 days. The content of monophenols in untreated stems of M 106 clone was slightly lower than in the M 26 clone. The ratio of monophenols to o-diphenols was significantly lower in untreated stems of M 106 and IBA treatment still decreased the ratio.

The activity of PO (Fig. 3) was much higher in the stems than in the leaves of the both clones tested (note the change in the scale units). IBA treatment caused changes of PO activity in leaves and stems of M 106 and M 26. It is difficult to sum up these changes, but it is notice-

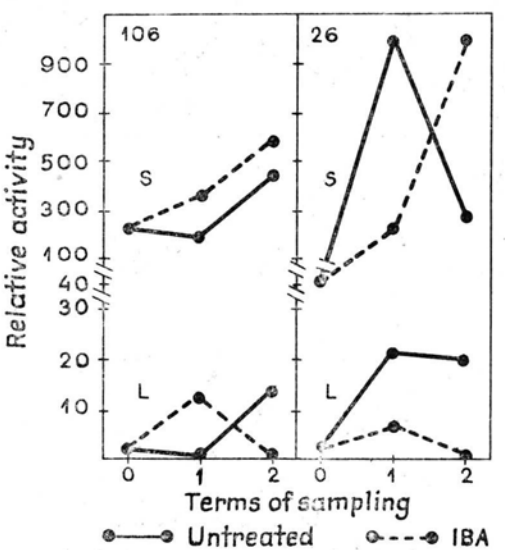

Fig. 3. The peroxidase activity in the leaves and stems of IBA-treated M 26 and M 106 softwood cuttings on day 0 of making cuttings and after 16 or 24 days of rooting. Denotations as for Fig. 2.

able that IBA caused a marked decrease of PO activity in the M 26 leaves and an increase in the M 106 stems.

There was no polyphenoloxidase activity detected in the samples tested.

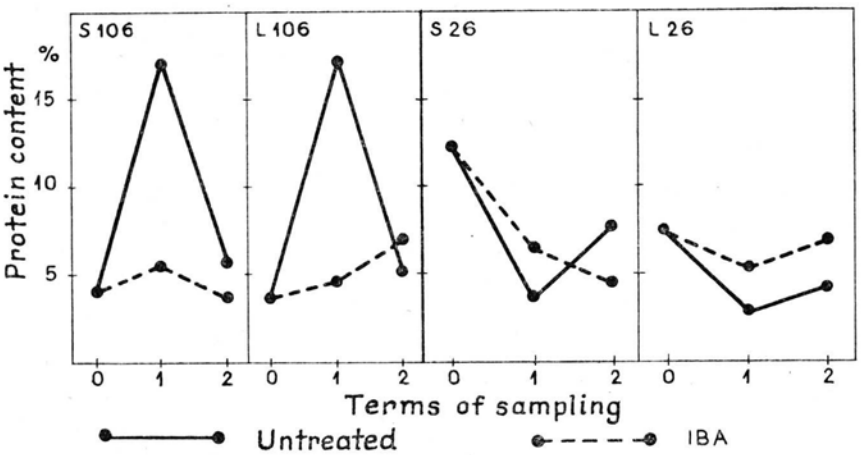

Fig. 4. Per cent of protein content in leaves' and stems' acetone extracts of IBA-treated M 26 and M 106 softwood cuttings on day of making cuttings and after 16 and 24 days of rooting. Denotations as for Fig. 2.

The content of protein in acetone powder extracts showed clearcut differences between M 26 and M 106 clones and univocal changes due to IBA treatment (Fig. 4). There was the same protein content in stems and leaves of M 106 clone but a higher protein content in the stems of M 26 at the beginning of the experiment. IBA caused a marked decrease of protein content both in the leaves and stems of M 106 and an increase in the leaves and stems of M 26 clone, after 16 days of rooting. In general, the cuttings from first experiment responded similarly in respect to the protein content.

DISCUSSION

It was found in the first experiment, that easier-to-root M 106 cuttings contained more promoting substances and contained less of an inhibitory cofactor at the R_f 0.8 (Fig. 1A). These differences could to some extent explain the rooting response of the clones compared, but it still remained unclear whether the content of rooting cofactors in the leaves or stems was important. The data from the next experiment were even more unclear in this respect (Fig. 1B) because the differences be-

tween M 26 and M 106 were not very pronounced and IBA treatment did not cause unidirectional changes in the responses of cuttings. The differences existing were rather of a quantitative than qualitative sort.

It is possible that the rooting inhibitor found at R_f 0.8 in apple cuttings is identical with these which have been found in pear cuttings by Fadl and Hartmann (1967), in tomato cuttings by Aung (1972) or in hibiscus cuttings by Stoltz and Hess (1966b). This compound isolated from apple cuttings has R_f 0.78—0.90 and is yellow under UV light +NaOH. In fact, some authors have proven correlations between rooting ability of cuttings and the content of rooting cofactors in different species (Hess, 1961; Ashiru Alaba, 1967; Fadl and Hartmann, 1967; Lanphear et al., 1963; Bojarczuk, 1978; Sadhu et al., 1978). The other authors, however, have found no such correlation (Lipecki and Dennis, 1970, 1972; Krzywański et al., 1976). Probably the results obtained by different authors were strongly influenced also by the time of sampling, as in this author's experiments.

Despite the doubts discussed above, it was proven that some phenolic compounds improved the rooting of cuttings when applied exogenously (Hess, 1968; Jankiewicz et al., 1973; Piątkowski et al., 1973; T. Bojarczuk and Jankiewicz, 1975) but were not effective when applied to difficult-to-root sour cherry cuttings (Lipecki et al., 1975).

As discussed elsewhere (Krzywański et al., 1976), there are cases in which exists an interrelation between the content of phenolic substances (rooting cofactors), enzyme activities and growth substances. From that point of view, the better rooting of M 106 cuttings could be partly explained by a much lower content of monophenols in the stems of IBA-treated cuttings of this clone which decreased monophenols (o-diphenols) ratio in M 106 much over that in M 26 clone (Fig. 2). The lower ratio of monophenols/diphenols was also found in the easy-to-root gooseberry cuttings (Krzywański et al., 1976). The activity of peroxidase measured in this experiment does not clearly fit a model presented by Basu (1971). However, more specific enzymes may have been involved or possibly isoenzyme patterns of PO were different for the clones and/or treatments compared. The protein content (Fig. 4) was much lower in both leaves and stems of M 106 IBA-treated cuttings after 16 days of rooting and in the M 26 stems only after 24 days. This may indicate a much faster response of M 106 cuttings to the treatments applied. Actually, perhaps even earlier stages of rooting should be considered.

Carbohydrates also play a role in the root regeneration of different species (Stoltz and Hess, 1966a; Górecki and Bors, 1976; Moore et al., 1972; Krzywański et al., 1976; Sandhu et al.,

1978). Unfortunately, carbohydrates were not assayed in the experiments discussed. A strong need exists for complex physiological investigations in order to more completely explain the physiology of adventitious root formation in cuttings.

REFERENCES

Ashiru Alaba C., 1967. Physiological studies of rooting cuttings of Malling-Merton 106 (MM 106) and East Malling II (EM II) apple (*Malus silvestris* Mill) clones. Ph. D. Thesis, Dept. Hort., Mich. State Univ., East Lansing, Michigan.

Aung L. H., 1972. The nature of root-promoting substances in *Lycopersicon esculentum* seedlings. Physiol. Plant. 26: 306-309.

Basu R. N., 1970. Indole acetic acid oxidizing system in relation to synergism and antagonism between auxins and non-auxinic chemicals in rooting of cuttings. Plant Physiol. 13: 250-262.

Basu R. N., 1971. Hormonal basis of regeneration of roots on cuttings. Indian Agric. 15: 69-85.

Bojarczuk T., Jankiewicz L. S., 1975. Influence of phenolic substances on rooting of softwood cuttings of *Populus alba* L. and *P. canescens* Sm. Acta agrobot. 28: 121-129.

Bojarczuk K., Jankiewicz L. S., 1975. Rooting of *Syringa vulgaris* L. softwood cuttings using auxin, vitamins, phenolic substances, SADH and abscisic acid. Acta agrobot. 28: 229-239.

Bojarczuk K., 1978. Anatomiczne i fizjologiczne badania sadzonek lilaków (*Syringa vulgaris* L.) w trakcie ich zakorzeniania. Arboretum Kórnickie 23: 101-120.

Bojarkin A. N., 1954. Bystryi metod opridielieniya aktivnosti peroksidazy. Biokhimiya 16: 352-356.

Fadl M. S., Hartmann H. T., 1967. Isolation, purification and characterisation of an endogenous root-promoting factor obtained from basal sections of pear hardwood cuttings. Plant Physiol. 22: 541-549.

Fehrmann H., Dimond A. E., 1967. Peroxidase activity and *Phytophtora* resistance in different organs of the potato plants. Phytopath. 57: 69-72.

Górecki R. S., Borys M. W., 1976. The effect of inorganic salts, sucrose and CEPA on rooting of willows' cuttings. Acta agrobot. 29: 43-49.

Górecki R. S., 1979. The effect of an auxin (IBA), fungicide (Captan) and of wounding on the rooting of softwood apple (*Malus* Mill.) cuttings. Acta agrobot. 32: 223-232.

Hess C. E., 1962a. Characterization of rooting cofactors extracted from *Hedera helix* L. and *Hibiscus rosa-sinensis* L. Proc. Plant Prop. Soc. for 1961: 51-57.

Hess C. E., 1962b. A physiological comparison of rooting in easy- and difficult-to-root cuttings. Proc. Plant Prop. Soc. 12: 265-268.

Hess C. E., 1964. Naturally occurring substances which stimulate root initiation. Reg. de la Crois. Veg. Paris: 514-517.

Hess C. E., 1968. Internal and external factors regulating root initiation. In: Root growth, Proc. 15th Easter School Agr. Sci., Univ. Nottingham, Ed. W. J. Whittington. Butterworths, London.

Jankiewicz L. S., Bojarczuk T., Piątkowski M. G., 1973. The effect of rutin and pyrogallol upon rooting of softwood cuttings of magnolias and of *Syringa meyeri* Schneid. Acta agrobot. 26: 277-283.

Johnson L. B., Schaal L. A., 1957. Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease resistance. Amer. Potato Jour. 34: 200-209.

Krzywański Z., 1975. Phenolic compounds in healthy and infected with *Didymella applanata* annual woody canes of three raspberry cultivars. (PL, En. summary), PTPN, Pr. Komis. Nauk Rol. i Komis. Nauk Leś. 39: 157-164.

Krzywański Z., Górecki R. S., Czech M., 1976. Some physiological properties of easy-to-root gooseberry (*Ribes grossularia* L.) tumours. Marcellia 39: 107-112.

Lanphear F. O., Meahl R. P., 1963. Influence of endogenous rooting cofactors and environment on the seasonal fluctuation in root initiation of selected evergreen cuttings. Proc. Amer. Soc. Hort. Sci. 83: 811-818.

Lipecki J., Dennis F. G., 1970. Kofaktory ukorzeniania się sadzonek. Wiad. bot. 14: 141-149.

Lipecki J., Dennis F. G., 1972. Growth inhibitors and rooting cofactors in relation to rooting response of softwood apple cuttings. Hort. Sci. 7: 136-138.

Lipecki J., Selwa J., Blamowski Z., Grzybowska D., 1975. Effect of some growth regulators on the rooting of black currant, sour cherry and forsythia softwood cuttings. Fruit Sci. Rep. 1: 55-64.

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Mast C. A., van der 1970a. Separation of IAA degrading enzymes from pea roots on columns polyvinylpyrrolidone. II. Acta Bot. Neerl. 19: 141-146.

Mast C. A., van der 1970b. The inhibiting effect of caffeic acid on the enzymatic degradation of IAA peroxidases. Acta Bot. 19: 659-664.

Moore K. G., Cobb A., Lovell P. H., 1972. Effect of sucrose on rooting and senescence in detached *Raphanus sativus* L. cotyledons. J. Exp. Bot. 23: 65-74.

Piątkowski M. G., Jankiewicz L. S., Kasprzyk S., 1973. Use of auxin, fungicides and rooting cofactors to induce adventitious root formation in softwood cuttings of apple, gooseberry and some ornamental plants. Acta agrobot. 26: 191-201.

Poapst P. A., Durkee A. B., 1967. Root-differentiating properties of some simple aromatic substances of the apple and pear fruit. J. Hort. Sci. 42: 429-438.

Psenak M., Jindra A., Kovacs P., 1970. Simple phenolic glycosides as potential regulators of the IAA-oxidase system. Biol. Plant., 12: 241-245.

Sandhu M. K., Sanghamitra Bose., Saha L., 1978. Auxin synergists in the rooting of mango cuttings. Sci. Hort. 9: 381-387.

Sirois J. C., Miller R. W., 1972. The mechanism of the scopoletin-induced inhibition of the peroxidase catalyzed degradation of indole-3-acetic acid acetate. Pl. Physiol. 6: 1012-1018.

Stoltz L. P., Hess C. E., 1966a. The effect of girdling upon root initiation: carbohydrates and amino acids. Amer. Soc. Hort. Sci. 89: 734-743.

Stoltz L. P., Hess C. E., 1966b. The effect of girdling upon root initiation: auxin and rooting cofactors. Amer. Soc. Hort. Sci. 89: 744-751.

Swain T., Hillis W. E., 1959. The phenolic constituents of *Prunus domestica* L. The quantative analysis of phenolic constituents. J. Sci. Food Agric. 1: 63-68.

Fizjologiczne zmiany w czasie ukorzeniania się sadzonek zielnych podkładek jabłoni (*Malus* sp. Mill.) M 26 i M 106

Streszczenie

Porównano poziom zawartości kofaktorów ukorzeniania, monohydroksyfenoli i o-dwuhydroksyfenoli, sumy fenoli i białka oraz aktywności peroksydazy i oksydazy polifenylowej w liściach i łodygach sadzonek zielnych podkładek jabłoniowych M 26 i M 106. Materiał do analizy wzięto w momencie zakładania doświadczeń oraz po 16 i 24 dniach ukorzeniania, u sadzonek kontrolnych i u sadzonek traktowanych kwasem 3-idolilomasłowym (IBA) w ilościach 5 mg w 1 g mieszaniny talku i Kaptanu (w stosunku 6:4). Znaczne różnice stwierdzone w zawartości wymienionych substancji nie tłumaczą dostatecznie ani lepszego ukorzeniania się sadzonek M 106, niż sadzonek M 26, ani stymulującego wpływu IBA na ukorzenianie się obu badanych podkładek. Jedynie niższa zawartość monohydroksyfenoli w łodygach sadzonek M 106 traktowanych IBA wyraźnie korelowała z lepszym ukorzenianiem się tej podkładki.