weeks later. The precision scoring crew of 15 to 18 people worked at 4 distinct times — a total of 5 weeks.

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FINGERPRINTING APPLES: A CHEMICAL METHOD OF IDENTIFYING CULTIVARS

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Abstract: To identify apple, *Malus pumila* Mill. [syn. M. *domestica* Borkh], cultivars, electrophoretic separation of proteins and isozyme patterns from shoot bark extracts was investigated. Cross-examination of enzyme banding patterns allowed the identification of 33 clonal apple rootstocks. Virus-tested rootstocks were distinguishable from the original contaminated material, and selections of 2 rootstocks propagated by tissue culture expressed rather broad isozymic differences compared with their respective original stocks. Of 57 clonal apple scion cultivars and sports, all cultivars were identified. Sports within each cultivar, however, were indistinguishable, with the exception of 'Wijcik', a natural compact mutant of 'McIntosh'. Isozymic patterns of scion cultivars showed no apparent effect of sample timing, rootstock, growing location, or age of the wood where the sample was taken.

A precise method of identification of tree fruit cultivars is needed, especially during the early vegetative growth phases and during dormancy when many morphological criteria cannot be used. Tree fruit nurseries are especially interested in an identification method to help correct labeling errors or losses and to assist in patenting or with patenting infringement. Growers need a technique to positively identify suspected scion and rootstock errors in their own plantings or to establish true identities where orchard purchases have been made or are anticipated. A chemical identification method has the potential to be more precise than the usual morphological approaches to identification.

Chemical taxonomy, that is, the classification of plants based on chemical differences, is not a new method. It has been used to help establish taxa, genera, species, and ecotypes and is an invaluable aid in evolutionary studies (3). Many chemicals have been used as so-called genetic markers, i.e. oils in citrus, resins in conifers, and phenolic compounds in many plants. More recently, extensive work has been done with proteins, and more specifically enzymes. Proteins provide

some advantages over other compounds since the techniques may be less involved, faster, more economical and more positive, and proteins are less affected by environmental conditions (1). Proteins are stable and reliable for identification purposes, but the same type of tissue should be compared for identification, and the sample should preferably by taken at the same stage of growth.

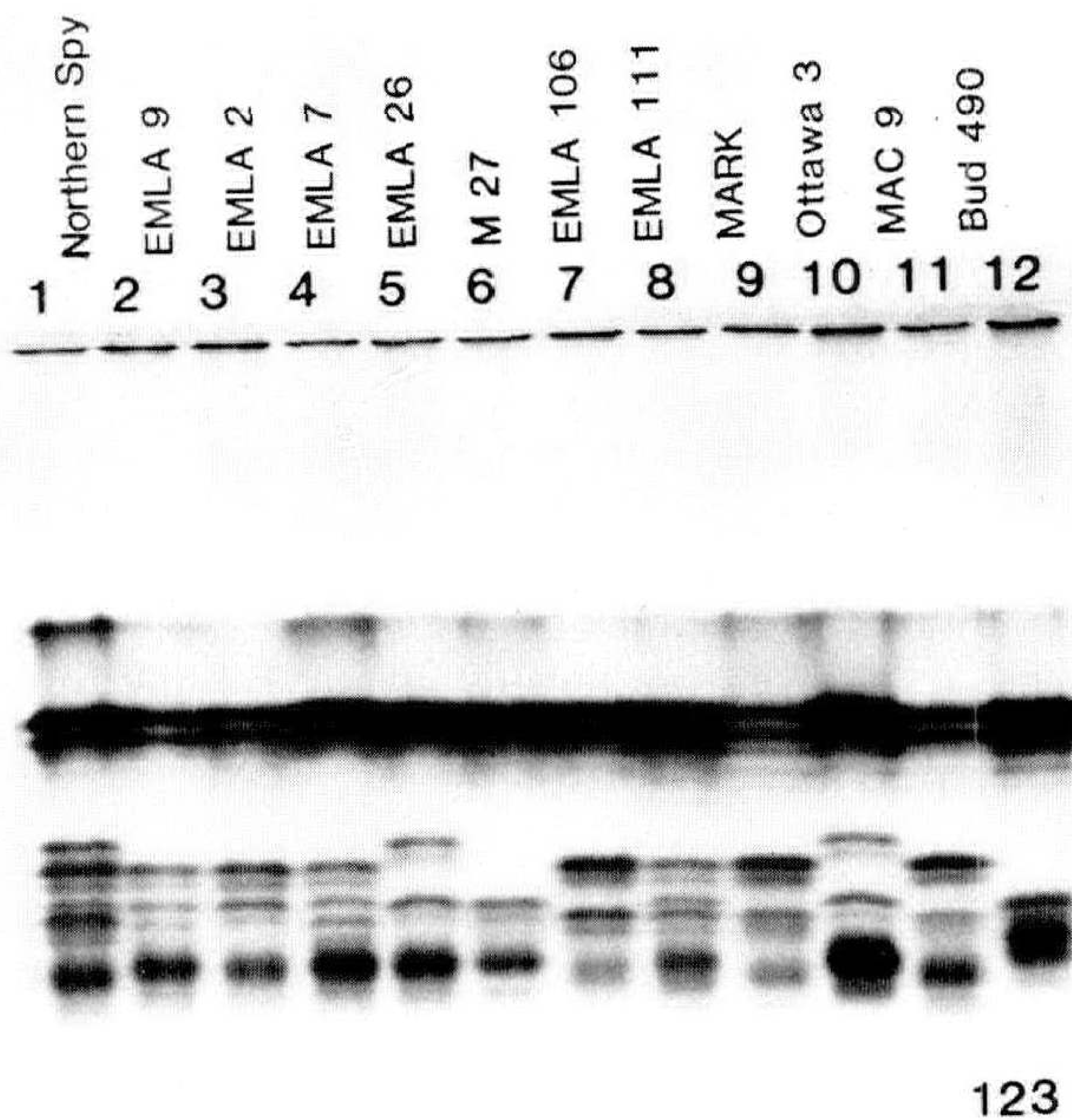
A physico-chemical process called electrophoresis can separate proteins in a plant extract on the basis of their electric charge and/or size. The protein extract can be made from leaf, stem, or root tissue. After the proteins are separated in a gel matrix, proper staining will reveal the position of the proteins in the form of bands in the gel. The results can either be recorded photographically or by densitometric scanning.

In order for a fingerprinting technique to be useful, it must satisfy several requirements:

- It must be based on constant markers; the constancy of the markers must hold for samples taken from different growing areas and at different times of the year.
- The methodology should be reproducible; different laboratories should be able to follow the procedures and obtain identical results.
- It should be able to identify clones at a time when morphological or horticultural characteristics are not observable (i.e. young trees, dormant season, interstems where no leaves are present, etc.)

In working with proteins, it is more useful to look at specific enzymes in their different forms (isozymes). Specific staining techniques are available for many enzymes (2). In apple clones, we have found several enzymes extracted from shoot bark tissue, that contain many forms, or isozymes, and these are very useful for identification of many differences found among cultivars (Figure 1). Because clones are asexually propagated, a difference in banding patterns for a given enzyme between two cultivars is proof of genetic dissimiliarity. Two cultivars differing in one or more enzyme banding patterns can be readily separated. Not all enzymes have a different pattern in every cultivar. For many, the patterns are common. It is the cross-examination of different patterns for several enzyme systems which gives fingerprints for each clone.

By repeated cross-examination we have been able to differentiate among 33 clonal rootstocks (Table 1). In Table 1 different banding patterns are identified by numbers within each enzyme/electrophoretic condition. Therefore, rootstocks with the same number, within a column, cannot be differenti-



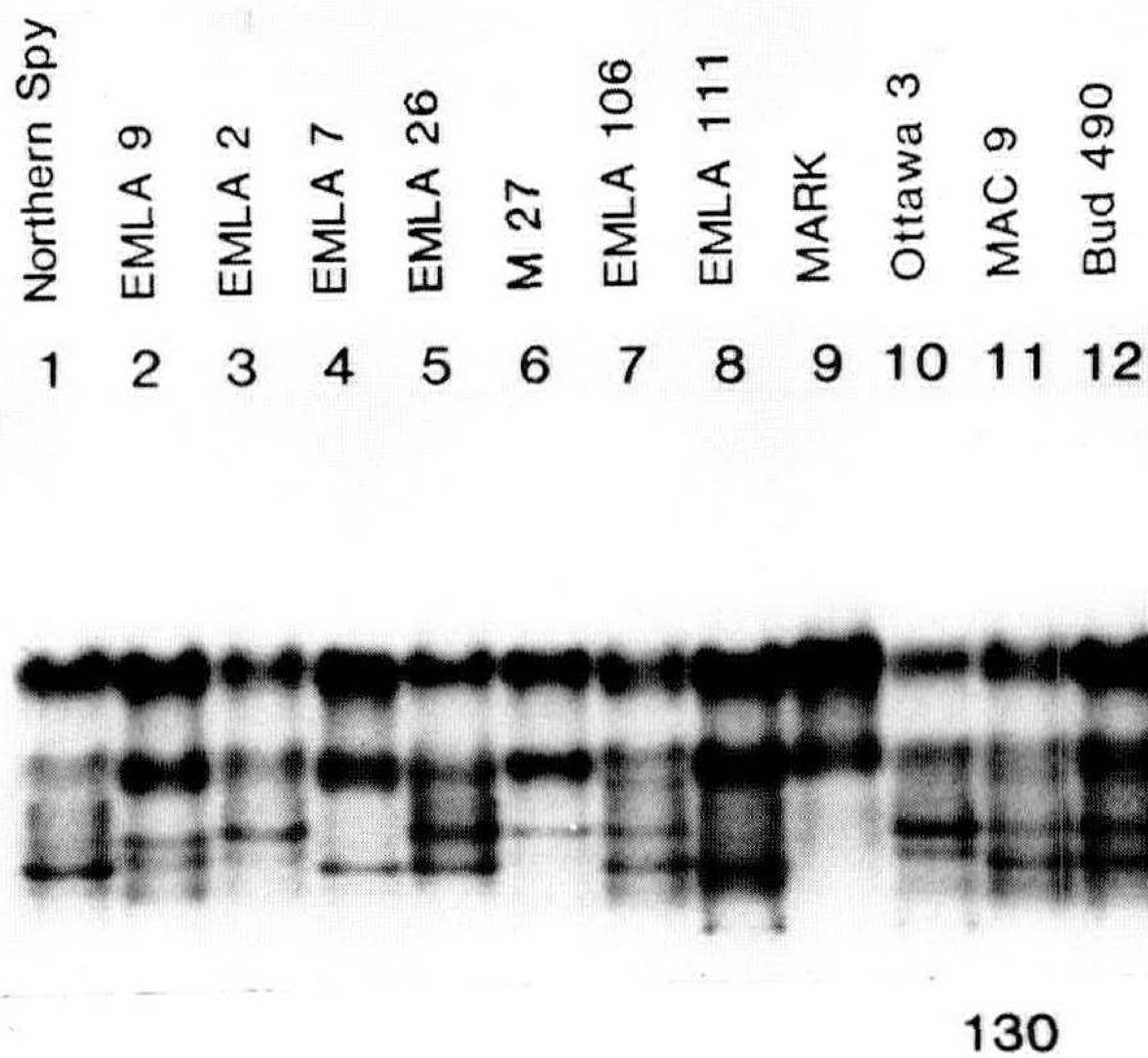


Figure 1. Polyacrylamide gels showing the banding patterns of 11 apple rootstocks. Each gel was stained for a different enzyme. The relative position of the bands, in the vertical direction under each rootstock, represents the fingerprint for that enzyme. Note how two rootstocks may have the same pattern in one gel, but can be distinguished by different patterns in the other gel (enzyme system). For example: EMLA 2 and EMLA 7 are the same in gel #123, but in gel #130 are easily differentiated.

ated by that enzyme system. For example, under enzyme "a", rootstock #2 (M 4), and rootstock #3 (M 9) each share banding pattern 2. Budagovsky 490, rootstock #28, is represented by banding pattern 6, which is a unique pattern for that rootstock within the "a" system. No differences in banding patterns were observed due to growing conditions, or when sampling was done at different times throughout the year.

The large number of banding patterns obtained for each enzyme indicates the great genetic diversity of clonal apple rootstocks, and suggests that several genes may be involved in the coding of so many different variants. Even clones with very close morphological traits, or those genetically related, possess very few isozymic similarities; for example, M 26, M 27, MAC 9, P 2 (Polish series), P 22, and Ottawa 3 all have M 9 as one parent, and yet, none of them share more than 3 patterns with M 9.

Virus-tested clones were distinguishable from the original contaminated material, and virus-tested clones from different programs (East Malling-Long Ashton and Washington Certified IR2 Project) expressed some isozymic differences. MAC 9 (Michigan series) and MARK (virus-tested MAC 9) were also very similar but distinguishable. The horticultural differences sometimes observed between the virus-tested material and their original stocks might be explained by genetic differences (possibly originating in the eradication or selection process) or by the inherent effects of the presence of viruses.

The tissue culture propagated selections of M 7 and MAC 9 expressed significant deviations from the mother clones, distinguishable by 7 of the 9 enzyme systems. These selections are indeed new clones, and their horticultural value should be carefully tested.

Table 2 lists all the scion cultivars and sports tested in this study. All cultivars included were positively identified by the cross-examination of the isozyme banding patterns of 4 enzymes. Sports within the same cultivar, however, appeared identical and were, therefore, indistinguishable. The only exception was 'Wijcik', a natural compact mutant of 'McIntosh', which could be distinguished from the latter but appeared indistinguishable from 'Spartan'. The banding patterns for each enzyme and scion cultivar are summarized in Table 3.

As with the clonal apple rootstocks, identification of apple scion cultivars was feasible by using their isozymic diversity. It was evident that each enzyme produced fewer patterns than found for the clonal rootstocks, indicating a more narrow genetic base.

Table 1. Isozyme patterns[z] produced by enzyme systems used to distinguish several clonal apple rootstocks. Northern Spy was used as a comparative standard.

Rootstocks	ENZYME SYSTEMS								
	a	b	c	d	e	f	g	h	i
1. Northern Spy	1	1	1	1	1	1	1	1	1
2. M 4	2	2	12	12	7	9	3	12	5
3. M 9	2	13	10	17	2	2	3	14	3
4. M 26	3	14	16	19	2	2	3	5	8
5. M 27	4	4	5	5	4	5	1	5	17
6. MM 106	5	17	6	22	3	14	7	4	6
7. MM 111	2	15	6	13	5	15	3	4	1
8. EMLA 2	2	2	3	2	13	3	1	2	16
9. EMLA 7	2	2	2	3	3	4	2	3	5
10. EMLA 9	2	2	2	2	2	2	2	2	15
11. EMLA 26	3	3	4	4	2	2	3	4	8
12. EMLA 104	5	12	19	16	11	6	3	3	14
13. EMLA 106	5	2	6	6	3	6	1	6	6
14. EMLA 111	2	2	6	7	5	1	4	8	1
15. M 9 WC IR2[y]	2	13	10	20	2	2	3	14	3
16. M 26 WC IR2[y]	3	6	10	11	2	2	3	3	8
17. MM 106 WC IR2[y]	5	17	6	21	3	14	7	4	6
18. MM 111 WC IR2[y]	2	15	6	1	5	15	3	4	1
19. M 7 TC[x]	2	13	4	13	12	2	3	13	5
20. MARK	5	2	7	8	2	14	1	7	18
21. MAC (Michigan series) 1	9	2	15	16	6	12	2	10	13
22. MAC 9	5	2	7	8	2	14	1	6	19
23. MAC 10	1	1	6	10	6	1	3	9	9
24. MAC 46	4	4	19	14	9	11	5	8	13
25. MAC 9 TC[x]	5	12	18	18	2	5	3	15	4
26. Ottawa 3	3	3	8	9	4	7	1	12	4
27. Budagovsky 118	9	16	17	17	2	2	1	14	7
28. Budagovsky 490	6	5	9	10	2	7	1	6	3
29. Budagovsky 491	5	10	14	13	2	10	1	4	12
30. Antonovka 306	2	9	5	14	8	1	1	4	11
31. Antonovka 313	3	11	4	2	10	13	6	11	11
32. Polish series 2	7	7	11	11	2	8	3	10	4
33. Polish series 22	10	6	16	4	12	2	3	13	2
34. Cornell-Geneva 10	8	8	13	15	2	2	1	3	11

[z] Rootstocks within a column with the same pattern number are not distinguishable by that enzyme.
[y] Washington State certified virus free
[x] Tissue culture propagated selections of the original

Table 2. Apple scion and sport cultivars identified by isozyme analysis.

Delicious and sports	Miscellaneous cultivars
Starking Delicious	Imperial McIntosh
Wellspur	Roger's McIntosh
Red Chief	Wijcik
Hi-Early	Jonagold
Ace	Jonamac
Apex	Nured Winesap
Red King	Winesap
Oregon Spur II	Winter Banana
BM-62 (sport of Oregon Spur II)	Spur Winter Banana
Sharp Red	Law Spur Rome
Scarlett	Spartan
Atwood	Empire
Early Red One	Idared
Bisbee	Lodi
Ryan Red Improved	Cox's Orange Pippin
Spur Ryan Red	Mitsu
Early Brite	Priscilla
Cascade	Granny Smith
Brite And Early	Spur Granny Smith
Silver Spur	Rhode Island Greening
Classic	Tydeman's Red
Red Spur	Gravenstein
Top Spur	Cortland
Top Red	Golden Delicious
Real McCoy	Gold Spur
Starkrimson	Sundale Golden Delicious spur
Aomori	Criterion
	Firmgold
	Earligold

One desirable objective, identification of sports within cultivars, could not be achieved by these methods. Even though some commercially named sports may in fact be genetically identical, the technique failed to distinguish between spur and non-spur types as well as between very early coloring 'Delicious' sports and the original 'Starking Delicious'. The chimeral nature of most sports apparently did not affect the genetic base of the enzymes most commonly used for cultivar fingerprinting.

With the exception of distinguishing between sports within cultivars, the methods for fingerprinting apples described here can be used by the nursery or fruit industry whenever the identity of a lot of trees is questioned. The procedure can be applied to young budded trees and can be used at any time of the year (during the growing season or when nursery trees are in storage after digging). A method of identification of sports or strains within a cultivar remains to be developed.

This report was based on work done by Ricardo Menéndez to fulfill the requirements for a Ph.D. degree at Washington

Table 3. Banding patterns[z] produced by enzyme systems used to distinguish apple scion cultivars. Northern Spy was used as a comparative standard.

Cultivar	Enzyme Systems			
	a	c	f	g
Northern Spy	1	1	1	1
Delicious	2	2	2	1
Golden Delicious	3	3	3	2
Jonagold	4	4	3	1
Priscilla	4	2	3	1
Jonamac	4	5	3	1
Mutsu	4	4	4	3
Spartan, Wijcik	2	6	4	2
McIntosh	2	6	4	1
Empire	2	7	4	1
Idared	5	5	2	1
Winesap	2	8	2	1
Lodi	6	9	2	1
Granny Smith	7	10	1	3
Rhode Island Greening	1	10	1	1
Tydeman's Red	8	2	2	1
Gravenstein	8	2	1	2
Cortland	1	9	2	1
Winter Banana	4	7	2	1
Law Spur Rome	8	9	2	1
Cox's Orange Pippin	1	9	1	1

[z] Cultivars within a column with the same pattern number are not distinguishable by that enzyme.

State University. Partial financial support was given by central Washington tree fruit nurserymen, Washington State Nursery Association, the Horticultural Research Institute, Oregon Rootstock, Inc., and the International Dwarf Fruit Tree Association.

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VOICE: Question for Jim Will. What fraction of Armstrong's rose production is currently micropropagated? And could you give us some idea of the economics of micropropagation vs. field grafting of roses?

JIM WILL: We are now producing about 7½% of our roses by micropropagation. The cost of micropropagating roses is about twice that of field production. But we have many cultivars that cannot be produced by field-budding techniques. In the future these will be produced by micropropagation. Those, such as 'Mr. Lincoln' and 'Queen Elizabeth', which are easily field-produced, will not be micropropagated.

PHIL BARKER: In propagating different cultivars by micropropagation do you find distinct differences in the root system of these cultivars?

JIM WILL: We have more significant differences in shoot development in cultivar variation. With roses there is no particular difference in root development.

PHIL BAKER: Are the root systems in micropropagation different that what they are in conventional propagation — using the same cultivar?

JIM WILL: There is very little difference in the root system between a micropropagated and a seedling-grown rose.

BRUCE BRIGGS: In rhododendrons, the root system of a tissue-culture propagated plants is usually much heavier than a cutting-propagated plant, because of the way the tissue-culture plant develops — somewhat similar to a seedling grown plant, more of a mass of roots.

CHARLES TUBESING: A question for Jim Will. Do you encounter any viruses in your rose stocks?

JIM WILL: We use only stocks that have been heat-treated and viruses-indexed at the University of California, Davis, for propagation in tissue culture. Viruses have been one of the significant problems in greenhouse roses throughout the industry. Virus expression will appear in tissue-cultured material if clean stock is not used.

OPTIMIZATION OF TISSUE CULTURE MEDIA

CAROLYN ALBRECHT

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When Murashige and Skoog developed their medium for plant tissue culture over 20 years ago, it was seen to be a great breakthrough in *in vitro* plant propagation. By varying the concentrations of various plant growth regulators, researchers found they could grow a wide range of herbaceous plant species, plus some woody species.