Chris Wheatley

Centro Internacional de Agricultura Tropical

Serie SE-16-80
November 7, 1980

STUDIES RELATED WITH THE NATURE OF POST-HARVEST PHYSIOLOGICAL DETERIORATION IN CASSAVA ROOTS

Christopher Wheatley

RESUME

Post-harvest physiological deterioration of cassava roots is a serious limiting factor affecting storage over even short periods of time. The resulting black pigments render the roots inedible to both humans and animals. A better understanding of the causes of this problem and methods of control were the principal aims of the current investigation.

Using a simple evaluation procedure the variation within and between cultivars as regards the susceptibility to deterioration was found to be considerable. Pruning plants prior to harvest was confirmed to induce a resistance to deterioration in susceptible cultivars as well as lead to some reduction in root starch content. This was

found to continue for considerable periods after pruning(> 9 weeks). Ecosystem studies show that there is a large environmental component in the variation in deterioration susceptibility within one cultivar and that physiological stresses in general (climatic, biotic or edaphic) can lead to a reduction in susceptibility. Further data on harvests in Popayán support this.

Biochemical studies provided evidence for the existance of a phenol (scopoletin) which fluorescences blue in UV light, appears in fresh tissue a few hours after harvest and rapidly accumulates. Applications of scopoletin to fresh tissue induce blue-black vessel pigmentation within 6 hours. Tissues from pre-pruned (resistant) plants do not accumulate scopoletin to the same extent but respond to the exogenous compound. An enzyme inhibitor presents scopletin accumulation suggesting that its appearance is an enzymic process.

Further investigation into a combination of cultural practices and appropriate storage techniques with regard to root quality should enable the goal of successful cassava root storage to be achieved.

STUDIES RELATED WITH THE NATURE OF POST-HARVEST PHYSIOLOGICAL DETERIORATION IN CASSAVA ROOTS

by

Christopher Wheatley*

Introduction

Physiological deterioration of cassava (Manihot esculenta Crantz) roots was first recognised as such by Booth (Booth, 1976) who distinguished between primary (i.e. physiological) deterioration and the later secondary (microbiological) decay. The physiological reaction shows two distinct types of symptoms; a blue-black pigmentation of the vessels and brown-white dehydrated regions (especially frequent near damaged areas) in a "ring" around the periphery of the parenchyma. This deterioration can appear as soon as 2 days after harvest in roots which are very susceptible. Most roots become unacceptable for human consumption some 4-7 days after harvest. Microbiological decay, which can also produce the same blue-black vessel pigmentation (but without any pattern), generally occurs only a week or more after harvest or during storage in humid conditions. Several workers have noted differencies between cultivars as regards their susceptibility to physiological deterioration (Montaldo,1973; CIAT, 1976; Marriott,1978) and have devised evaluation methodology. A relationship between root durability and root dry matter content has also been noted (CIAT,1976, 1979).

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*Graduate student, Wye College, London University, London, England/Visiting Research Associate,CIAT; sponsored by Tropical Products Institute, London, England.

The inability to store cassava roots for any length of time has a profound effect on the harvesting and marketing practices of the crop. Fields of cassava grown for local consumption are not harvested at one time, but rather as and when required. This unavoidable practice keeps substantial areas of land essentially out of production; and, as root quality decreases with advancing age of a crop past maturity, the available starch declines in these plants.

An understanding of the physiological processes which cause the root discoloration as well as more information on the variation encountered in the field within and between cultivars could thus be of great importance towards the aim of storing cassava for substantially longer periods of time after harvest.

Methodology of field evaluation

The method essentially as described by Marriott(1978) was used in all field evaluations. Roots were selected using the following criteria:

1. minimal mechanical damage
2. medium size, $\geq$ 15cm in length
3. absence of any pre-harvest root-rot or decay

The roots were cut transversely at both head (proximal) and tail (distal) ends and a piece of thin PVC film secured tightly around the distal end with an elastic band. Roots were stored in ambient or laboratory conditions for 3 days before evaluating. Deterioration occured first at the exposed proximal end and progressed down the length of the 15cm test piece

with time. The evaluation was made by scoring, from 0 to 10 on a subjective visual scale, root sections cut at 2cm intervals down the root length- 7 sections in all. A "0" score signified no deterioration; "10", total deterioration and "5", that 50% of the circumference had deteriorated. The circumference and not the area was taken as the basis for evaluation because deterioration forms a ring of affected tissue around the edge of the cortex, leaving the centre unaffected. Normally 10 roots were scored per evaluation, the scores at each depth being averaged and these mean depth scores then averaged to produce one score per 10 roots, which was converted to a percentage. This deterioration percentage (Det.%) was used as a standard measurement throughout.

This method has several advantages over the present CIAT methodology for obtaining "root durability" by cutting up roots stored in paper bags after 2,4,6 and 8 days and scoring each root from 0-4.

(1) deterioration commences at sites of mechanical damage. Roots not standardised at harvest will have varying degrees of mechanical damage, not only along their length but most importantly at the two ends. The distal end, often damaged at harvest; and the proximal, of varying length, will vary considerably between roots. By cutting both ends and covering one, simulating a no-damage situation, this source of variation is removed.

(2) storage in bags can produce humidity changes which affect physiological deterioration.

(3) the single evaluation at 3 days, by which time substantial deterioration can develop, is much rapider than the alternative. Consequently there is less chance of confusion with microbiologically - induced deterioration which can develop during the longer periods of storage before evaluation.

Varietal Evaluation in CIAT

Cultivars have been previously evaluated in CIAT (from the germplasm collection) and a considerable range in susceptibility found. For detailed studies in the present work a few cultivars were selected.

M Col 22 - susceptible M Col 1684 - resistant

Llanera - moderately susceptible

M Col 113- moderately susceptible CMC 40 - tolerant

Figure 1 shows the results of all evaluations carried out in CIAT 1979-1980 with these five cultivars. Considerable variation within a cultivar was found, which makes the descriptions of varietal susceptibility and resistance rather doubtful. M Col 22, on which 50 evaluations were performed, was normally susceptible, but has been encountered with as low as 18% or 22% deterioration, meriting the description of tolerant. Similarly, the "resistant" cultivar, M Col 1684 has been evaluated at 1% and 62%. These general findings suggest that a genetically predetermined resistance or susceptibility to deterioration by itself is not sufficient to account for the amount of deteriora-

tion encountered, but that other factors such as environmental conditions may modify this genetic predisposition.

Pruning

Work at CIAT (Lozano et al, 1978) has previously shown that the pruning of plants a few weeks before harvest can dramatically reduce their susceptibility to deterioration. Further studies were conducted using M Col 22 with the aim of understanding the kinetics of the pruning effect in greater detail. Lozano et al reported that removal of all shoot regrowth was necessary to obtain the reduction in deterioration. Fig.2 shows that, where various treatments produce different quantities of regrowth 1 week after pruning, the reduction in Det.% is greatest in the treatment with the least amount of regrowth (prune + gramoxone). However, after two weeks the differences between treatments had decreased considerably and by three weeks the prune+gramoxone treatment, with only a 0.32 of regrowth had essentially the same Det.% as the prune-only treatment (392g regrowth). The suppression of shoot regrowth produces an equivalent but more rapid reduction in deterioration than in plants allowed to produce foliage at will. The production of foliage, however, must involve the mobilisation of the starch reserves of the roots until such time as the foliage reaches a size to become self sufficient in carbohydrate production. Foliage suppression, whether by chemical or manual means, should therefore help to maintain the root starch content.

Figure 3 shows the results of an experiment designed to

demonstrate this, as well as show the kinetics of the fall in Det.% in more detail. One month (32 days) after pruning the gramoxone-treated plants had less regrowth (36g to 876g) and more starch (32% to 31%) than untreated pruned plants. By two months (64 days) however, the gramoxone effect had largely worn off; regrowth had increased and starch decreased considerably. In both prune treatments Det.% had increased but slightly. The reduction in Det.% thus seems longer than previously thought.

Seven cultivars pruned (regrowth permitted) and evaluated (specific gravity method) for their starch content as well as deterioration at 0 and 9 weeks pruning show a similar picture. (Fig.4). An additional Det.% evaluation at two weeks demonstrates how rapid the fall in susceptibility can be. Starch content fell over the 9 week period in 6 of the 7 cultivars; but, significantly in CM 305-22 it rose (i.e. recovered) slightly without there being a corresponding rise in the Det.%. These data suggest that the relationship previously encountered between deterioration and starch (dry matter) content of roots (CIAT, 1976-1979) is not a causal one: the relationship holds up initially on pruning (both fall) but with time starch content will rise while deterioration may not necessarily do so.

Ecosystem variation

Evaluations of Det.% were made of the 25 cultivars in each site of the pathology section's ecosystem experiment. This trial enabled a study of the variation which exists between the ecosystems within each cultivar to be examined. Ten roots

per cultivar were evaluated at each ecosystem; occassionally (especially in Carimagua) not enough roots were produced for an evaluation to be carried out. Harvests were carried out at 12 months except for Popayán (15 months).

The complete results of the deterioration evaluations are in Table 1. The differences between the ecosystems are large and striking. In Carimagua and Media Luna the Det.% are almost zero for all cultivars while in Caribia they are only slightly higher. Only CIAT and Popayán show appreciable amounts of deterioration.

CIAT: M Col 22, several hybrids and the north coast cultivars of Secundina and Montero produced high Det.%. Several cultivars, however, had Det.% markedly lower than their "usual" scores (Llanera, 0.57%; CMC 40, 1.57%; M Col 113, 12.14%). M Col 1684 and M Ven 77 were resistant as expected.

Popayán: The local cultivars (Regional Amarilla and Negrita, CMC 92 and Sata Dovio) were susceptible and cultivars such as M Col 22, CM 305-120 were resistant (in CIAT, susceptible). M Pan 70, M Pan 19, CMC 344-71 showed much more deterioration than in CIAT whereas CM 305-122, (susceptible) and M Bra 12, M Col 1684 and M Ecu 82 (resistant) had similar Det.% to those in CIAT.

Carimagua: All cultivars with the exception of Montero were completely resistant (20 of the 22 cultivars had <1% scores). So resistant were these roots that after 6 days a further evaluation produced the same results.

Roots of freshly harvested M Col 22, M Col 113, CMC40, M Ven 77 and CMC 305-122 flown immediately after harvest to CIAT and stored in laboratory conditions (low relative humidity 60%, temp. 22°C) showed an identical resistance.

Media Luna: Only the local cultivar Montero deteriorated (8%) to even a small extent. The Det.%'s were only slightly higher than those of Carimagua.

Caribia: Again, a marked resistance was encountered. M Pan 19 at 28% was the highest score (although this may have been influenced by some fungal-induction of vascular streaking). The local cultivars of Secundina and Montero also produced some deterioration.

Relationship with starch content:

Figure 5 is a frequency distribution of the starch and deterioration % of each ecosystem. CIAT and Popayán with high starch and high Det.% in some cultivars show the expected pattern. Media Luna has a much lower content, and a correspondingly lower Det.% score. However, the starch% distributions of Carimagua and Caribia are not much lower than those of CIAT or Popayán and yet the Det.% scores are. Again, the suggestion is that the starch-deterioration relationship does not hold up under conditions were resistance to deterioration is encountered. Correlations coefficients obtained from these two parameters, for each ecosystem confirm this (Table 2). CIAT and Popayán have high values (0.1% significance) Caribia (10%) Carimagua and Media Luna (N.S.) do not.

Other Relationships:

Defoliation can be considered as a pruning treatment: the plant would be put under a considerable stress, starch reserves mobilised a new leaf production etc. It is therefore possible that plants defoliated may react in the same way as pruned plants as regards deterioration i.e. show a marked resistance. Plants in Carimagua were all almost completely defoliated prior to harvest by disease (CBB, superelongation and anthracnosis) mites, insects (lace bugs and lepidoptera) and by drought. Similarly, in Media Luna defoliation due to the prolonged drought was almost total, apart from the local cultivars. The dry season in Caribia, whilst not being as severe, was still sufficient in producing substantial defoliation. The local cultivars (Secundina, Montero) had the least defoliation and the highest deterioration %.

In CIAT, defoliation was moderate-low apart from Llanera (thrips) CMC 40 (root rots), cultivars which had surprisingly low Det.%'s.

Popayán, whilst having mod.-high defoliation and an unusually low rainfall did not have the expected intensity of Phoma.

Taken together, these results can reasonably be taken as evidence for a hypothesis that defoliation caused by climatic or biotic stresses leads to a reduction in susceptibility to physiological deterioration. It is possible that putting plants under a severe stress which is removed before defoliation occurs will be sufficient to induce resistance.

Experiments are in progress at present which should be able to answer these questions.

Further evidence is available:

		Det.%
1.	M Col 22 evaluated in Santander on plants defoliated due to drought stress :	15%
2.	Llanera in CIAT after an attack of thrips :	0%
3.	The susceptibility of a plot of M Col 113 decreased during the July - Sept. dry season in CIAT, 1980 :	June 26% Sept. 10%
4	An evaluation of CBB-attacked plants in Santander produced very low scores :	mean of cultivars 4%
5.	On evaluation of local cultivars at Popayán including those susceptible in the ecosystems harvests, all were resistant: all plants were badly affected by Phoma and hail damage. mean of cultivars :	8.2%

Popayán harvests in 1980

10 cultivars - 6 local and resistant to the NFP's of the ecosystem and 4 from other areas, were evaluated at the ICA

granja, Popayán in April, July and September of 1980. A harvest will take place in December. 3 local cultivars were also harvested in March. In general, cultivars were resistant in March, moderately so in April, less still in July and susceptible in September. The September data have a wide range of susceptibilities (Table 3) and correlations between deterioration%and several harvest characters (Foliage wt, production ha^{-1}, defoliation% and Starch%) all have significant values. This is because the local cultivars, well adapted to the cold climatic conditions of Popayán, have a high production of foliage and starch compared with the poorly adapted cultivars from other areas which are uniformly low in these characters. The Det.% agrees completely with this, the local cultivars having much more deterioration (mean 72.6%) than the non-local ones (mean 22.0%).

Selecting CMC 92 (local) CMC 39 (local) and M Mex 59 (non-local) and following the changes in the same parameters with time, the same story holds: (Fig.6) M Mex 59, with consistantly high defoliation and low production, has minimal deterioration whilst CMC 39, with low-moderate defoliation and high production has consistantly moderate-high deterioration. CMC 92, however, increased in Det.% through the year, and likewise increased in production ha^{-1}, foliage wt. and starch content and steadily decreased in defoliation.

Conclusion

All the available data suggest a relationship between the

susceptibility of a plant to deterioration and its ability to withstand the NFP's of a given ecosystem. The more severe the NFP's and the less adapted the plant is to withstand them, the further reduced will its deterioration be from its presumed inherent genetic susceptibility.

Biochemical Studies

It has been postulated (Lozano, 1978; Marriott, 1979) that a factor produced in the aerial part of the plant initiates the reactions which lead to the formation of the pigments associated with deterioration. The fact that complete pruning of plants induces resistance in the roots could be due to the progressive degradation of this factor and the absence of any replacement from the leaves or branches.

Initial studies to test this idea involved the placing of surface sterilised (to avoid contamination with microbial deterioration) deteriorated tissue in contact with fresh tissue. After incubation for 24hrs in the dark at 25°C, deteriorated vessels were observed only in the area of the fresh tissue in contact with the deteriorated tissue. (Table 4). A refinement of this method, in which the deteriorated and fresh tissue (cm^3 cubes of each) were separated by an agar block 2 or 5mm thick produced similar results (Table 5).

It was thus concluded that a diffusible substance was capable of passing from the deteriorated to the fresh tissues through the agar block. This substance was either the substrate or an inducer of the deterioration process.

Analysis (by TLC) of extracts from the agar blocks and from deteriorated tissues themselves showed the presence of a compound with a distinctive bright blue fluorescence in UV light. The Rf values obtained from TLC with various solvents and the UV absorbtion spectrum of the compound, as well as various specific spray reactions, showed the compound to be identical with a coumarin derivative called scopoletin as regards these properties. A more comprehensive analysis by Mrs. J. Rickard of the Tropical Products Institute has also shown this phenolic compound to be identical with scopoletin by HPLC and GLC.

Applications of commerical (Sigma Co. Ltd.) scopoletin to cubes of surface-sterilised root tissue produced rapid (12 hrs) and intensive discoloration of the vascular system as well as the surrounding parenchymal cells at concentrations of 500mg/1 and above (Fig.7). At 100mg/1 the effect was small. The relatively high concentration required for deterioration to occur, as well as the absence of UV fluorescence from totally deteriorated areas, suggests that scopoletin itself is the substrate of the reaction which leads to the pigmentation of vessels. Roots which are deteriorating fluoresce bright blue before any visible sign of discoloration appears. On the development of discoloration the fluorescence declines rapidly. Fresh roots fluoresce a dull purple colour.

Scopoletin applied to the tissue of pruned roots produces a similar amount of deterioration to the unpruned controls (Fig. 8). Pruned roots do not fluoresce to nearly the same

degree as unpruned, even 1 week after harvesting. This implies that pruned roots lack the mechanism by which scopoletin is formed or activated after harvest in normal roots.

Scopoletin applied to cured (in plastic bags at high humidities) roots, however, has a decreasing effect with storage time (Fig.9). Stored, resistant roots show significant amounts of light-blue fluorescence. This indicates that stored roots are capable of producing at least some scopoletin, but the mechanism of this subsequent oxidation to the presumed polyphenolic polymers is inhibited.

Applications of Cycloheximide, an inhibitor of protein (i.e. enzyme) synthesis, completely inhibits both deterioration and the appearance of blue fluorescence at concentrations of 100-1000 M (Fig.10) suggesting that the appearance of scopoletin is controlled by an enzymatic system.

An enzyme well known to have regulatory properties and to be involved in the biosynthesis of phenolics in potatoes, tomatoes and many other crops is phenylalanine ammonia lyase (PAL) (Camm and Towers, 1977) PAL catalyses the de-amination of the ammino-acid phenylalanine to trans-cinnamic acid and so starts the pathway of phenolic biosynthesis, resulting in the formation of scopoletin. PAL is known to increase rapidly in sweet potato tissue following infection and leads to the rapid production of phenolics including coumarins (Minamikawa,T., 1964). Phenol production is also stimulated by physiological stresses, e.g. metabolic poisons, in Yams (Uritani, 1959).

Despite repeated attempts no significant PAL activity could be detected in deteriorating tissue between 2hr and 3 days after harvest. It is therefore probable that scopoletin is either preformed in an inactive state in fresh tissue and enzymically activated after harvest or formed from other phenols which are present in fresh tissue.

Discussion and Conclusion

The results of the present work imply:

1. That it may be possible to devise cultural practices (pruning, total or partial) which effectively induce resistance to physiological deterioration without imparing greatly either the starch content or total yield.
2. That evaluations of root durability in breeding programs must take into account the condition of the plants evaluated. The genetic "potential" for susceptibility to deterioration will not necessarily be fully expressed in field conditions.
3. Resistant cultivars probably do exist, however, M Bra 12, moderately resistant (25-15%) in both CIAT and Popayán, also produced the highest yield in the CIAT harvest of the ecosystem experiment.
4. There are two ways of attacking the cassava storage problem: one is to prevent the accumulation of scopoletin (by pruning, etc.) the other is by inhibiting its oxidation to black pigments (by curing). A method which involved both aspects would stand the best chance of success.

REFERENCES

1. Booth, R.H. (1976). Storage of Fresh Cassava (Manihot esculenta Crantz). I: post-harvest deterioration and its control. Expl. Agric. 12:103-111.

2. Camm,E.L. and Towers, G.H.N. (1977). Phenylalanine Ammonia Lyase in Progress in Phytochemistry Vol.4. Eds. Reinhold; Harborne, J.B. and Swain, T. pp. 169-188.

3. CIAT, Annual Report (1976).

4. CIAT, Annual Report (1979).

5. Lozano,J.C.; Cock,J. and Castaño,J.(1978). Nuevos Avances en el almacenamiento de la yuca. Fitopatología Colombiana. 7:2-14.

6. Marriott,J.; Been,B.O. and Perkins,C. (1978). The Aetiology of Vascular Deterioration in Cassava roots after harvesting: Association with water loss from wounds. Physiol. Plant 44:38-42.

7. Marriott,J. (1979). The Aetiology of Vascular Deterioration in cassava roots after harvesting: Development of Endogenous Resistance in stored roots. Physiol. Plant 45:51-56.

8. Minamikawa,T. and Uritani,I. (1964). Phenylalanine deaminase and tyrosine deaminase in sliced or black rot infected sweet potato roots. Arch. Biochem. Biophys. 108:573-574.

9. Montaldo,A. (1973). Vascular Streaking in Cassava Root Tubers. Trop. Sci. 15(1): 39-46.

10. Uritani,I.; Uritani,M. and Yamada,H. (1959). Similar Metabolic Alterations induced in sweet potatoes by poisonous chemicals and by Ceratostomella fünbriata Phytopath. 50:30-34.

Fig.1

Frequency distribution of results of evaluations of five cultivars in 1979-80.

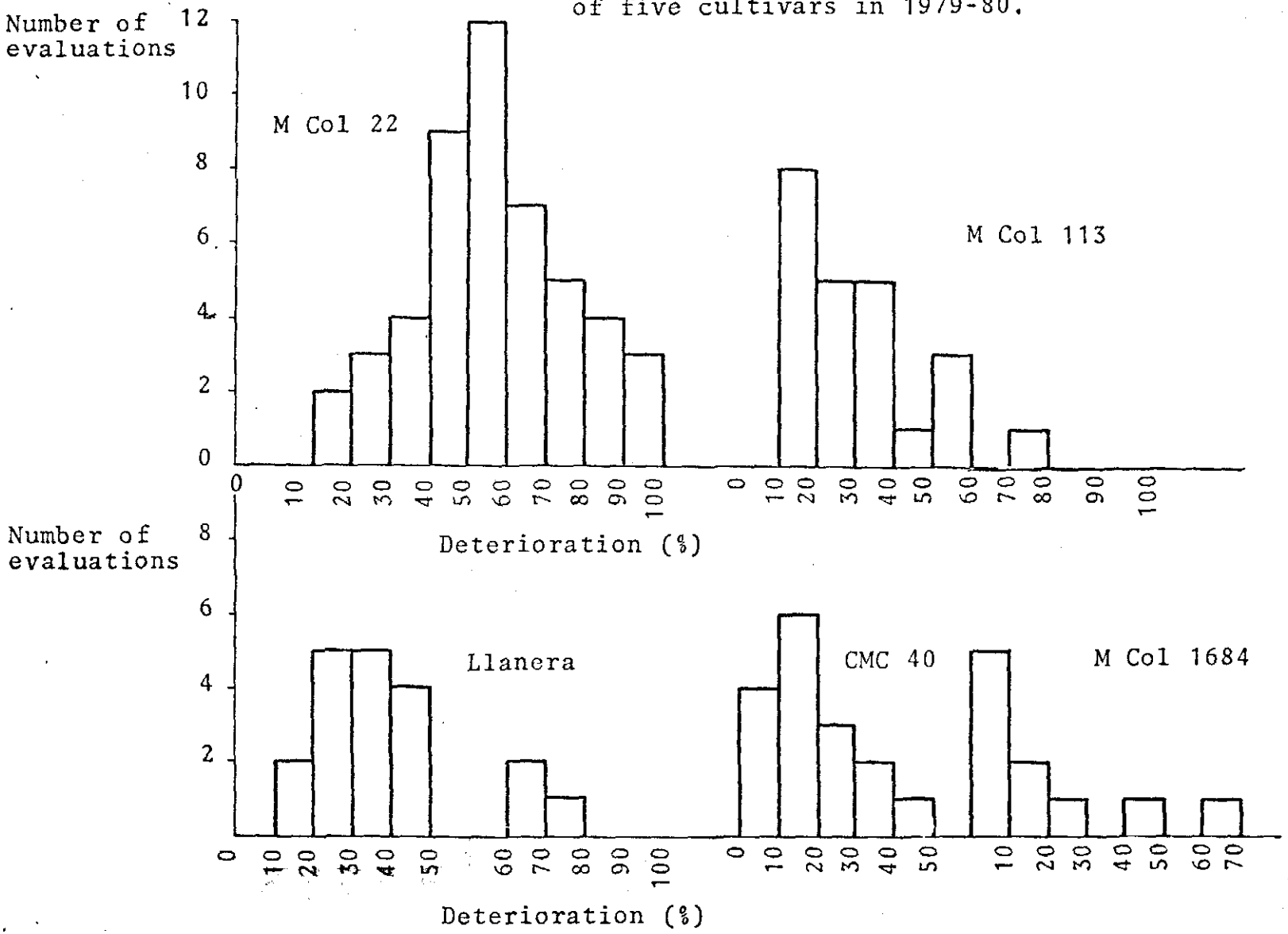


Fig.2 Regrowth and Deterioration in pruned M Col 22 plants.

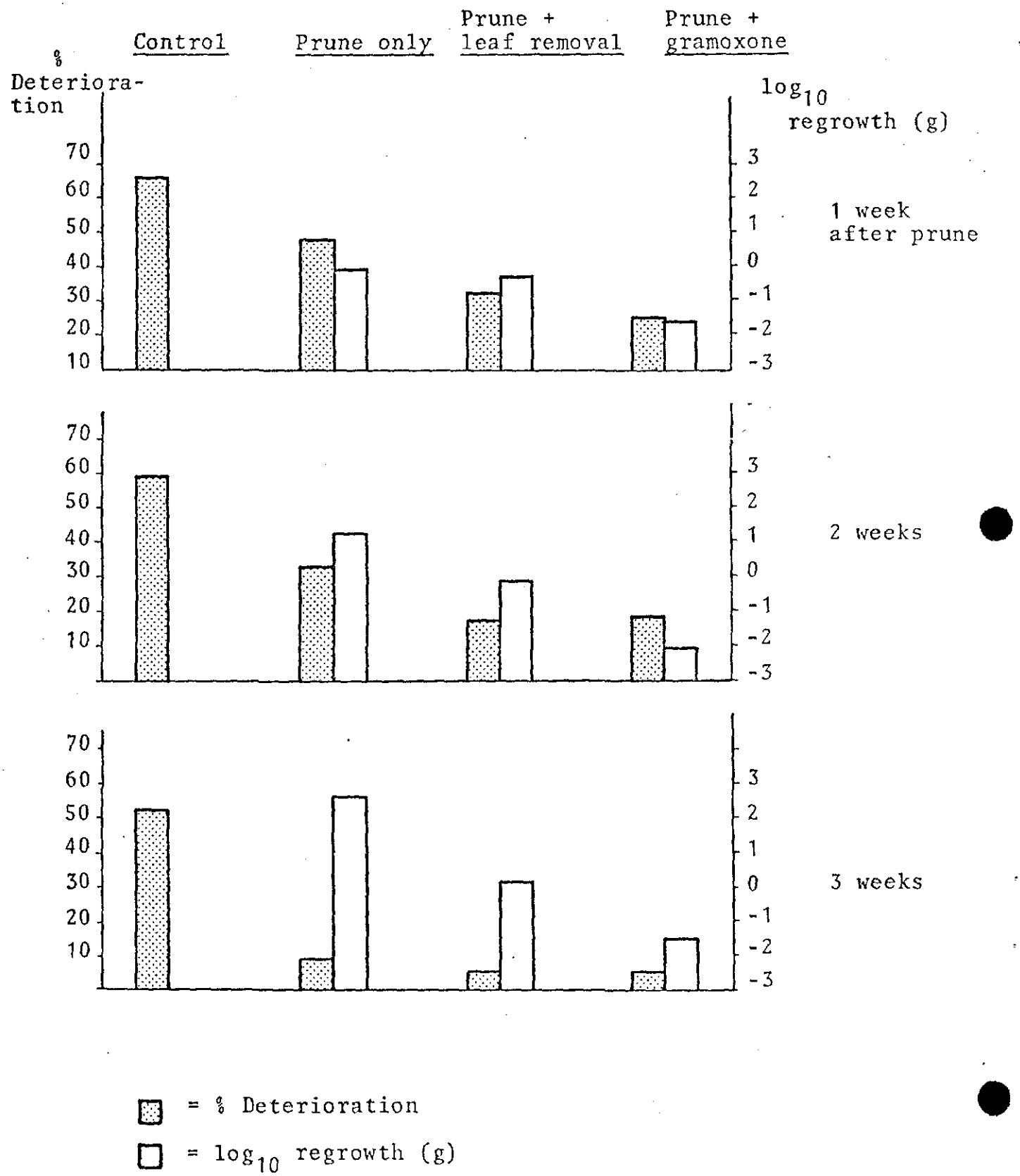


Fig.3 Deterioration, regrowth and starch content changes with time after pruning of M Col 22.

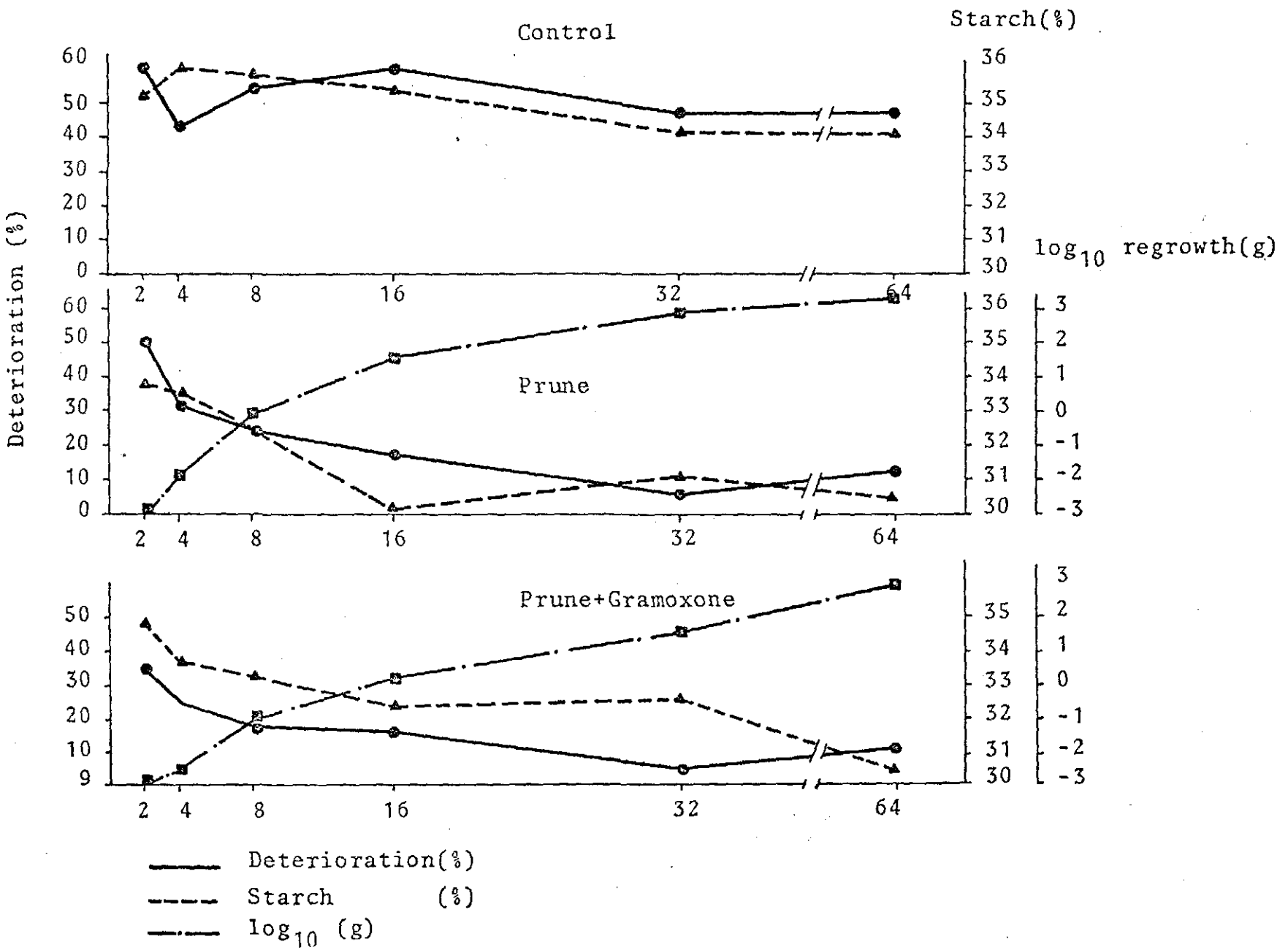


Fig.4

The effect on starch content and deterioration of pruning before harvest (regrowth permitted)

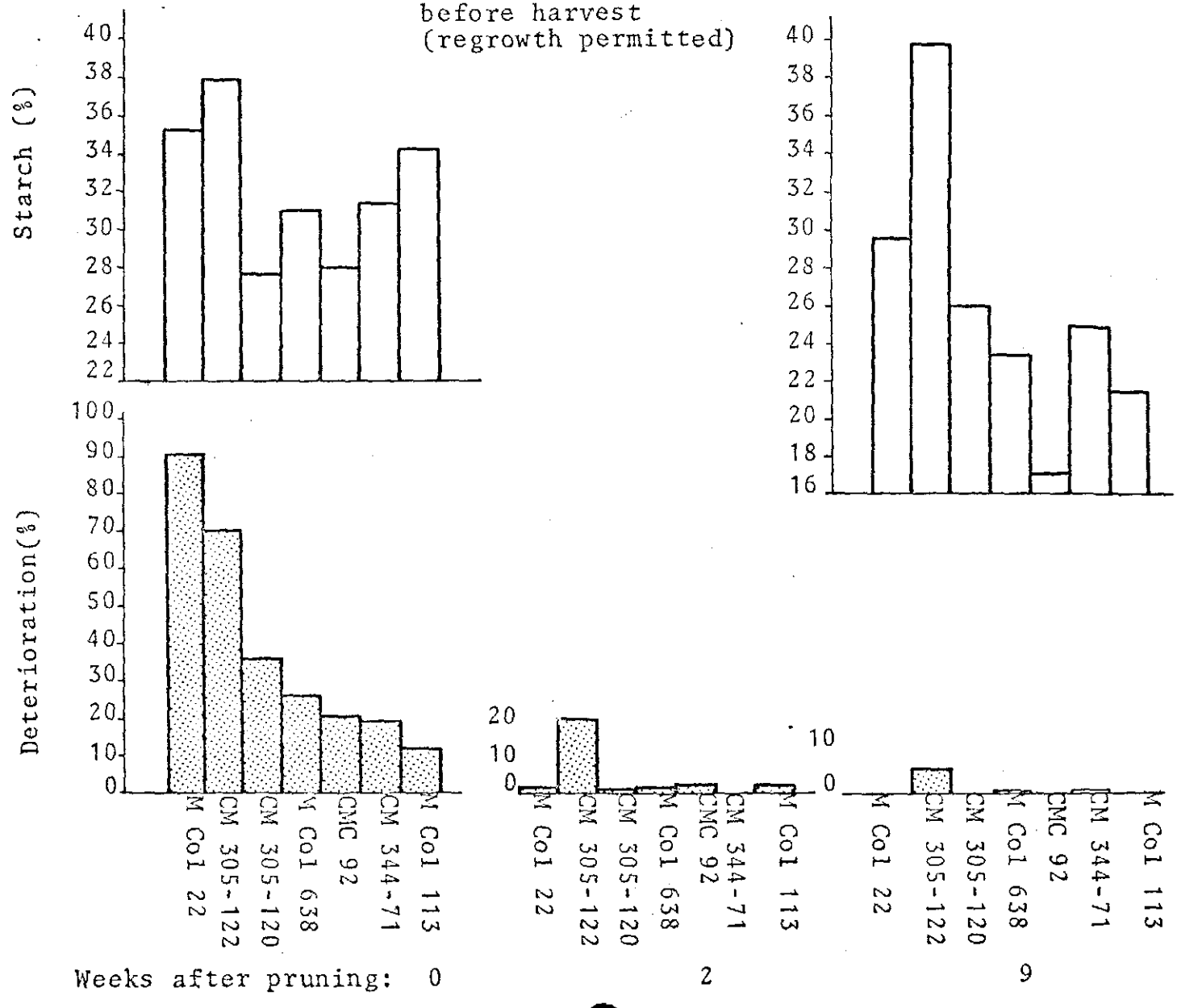


Table. 1. Deterioration (%) of 25 cultivars harvested in each of five ecosystems.

CULTIVAR.	CIAT	CARIMAGUA	MEDIA LUNA	CARIBIA	POPAYAN
CM 305-120	36	0	2	2	9
CM 305-122	70	0	2	3	63
CM 323-64	19	0	0	1	26
CM 340-30	29	0	1	1	14
CM 344-71	19	0	1	0	64
CMC 40	2	0	3	1	10
CMC 92	25	-	0	0	33
Llanera	1	1	1	-	2
M Bra 12	23	0	0	0	10
M Col 22	90	0	2	2	4
M Col 72	50	0	1	1	2
M Col 638	26	0	1	1	9
M Col 1684	13	3	1	7	4
M Ecu 82	8	0	0	2	4
M Pan 19	5	0	2	28	31
M Pan 70	15	0	1	1	57
M Pan 114	2	0	1	1	6
M Ven 77	3	1	1	7	25
Manteca	18	0	3	2	-
Montero	70	27	8	17	-
Reg.Amarilla	15	-	1	1	83
Reg.Morada	32	0	0	0	34
Sata Dovio	13	0	3	0	69
Secundina	59	-	2	24	-
M Col 113	12	0	4	0	34

Fig.5

Deterioration % and Starch % frequency distributions in each of five ecosystems harvested in 1980.

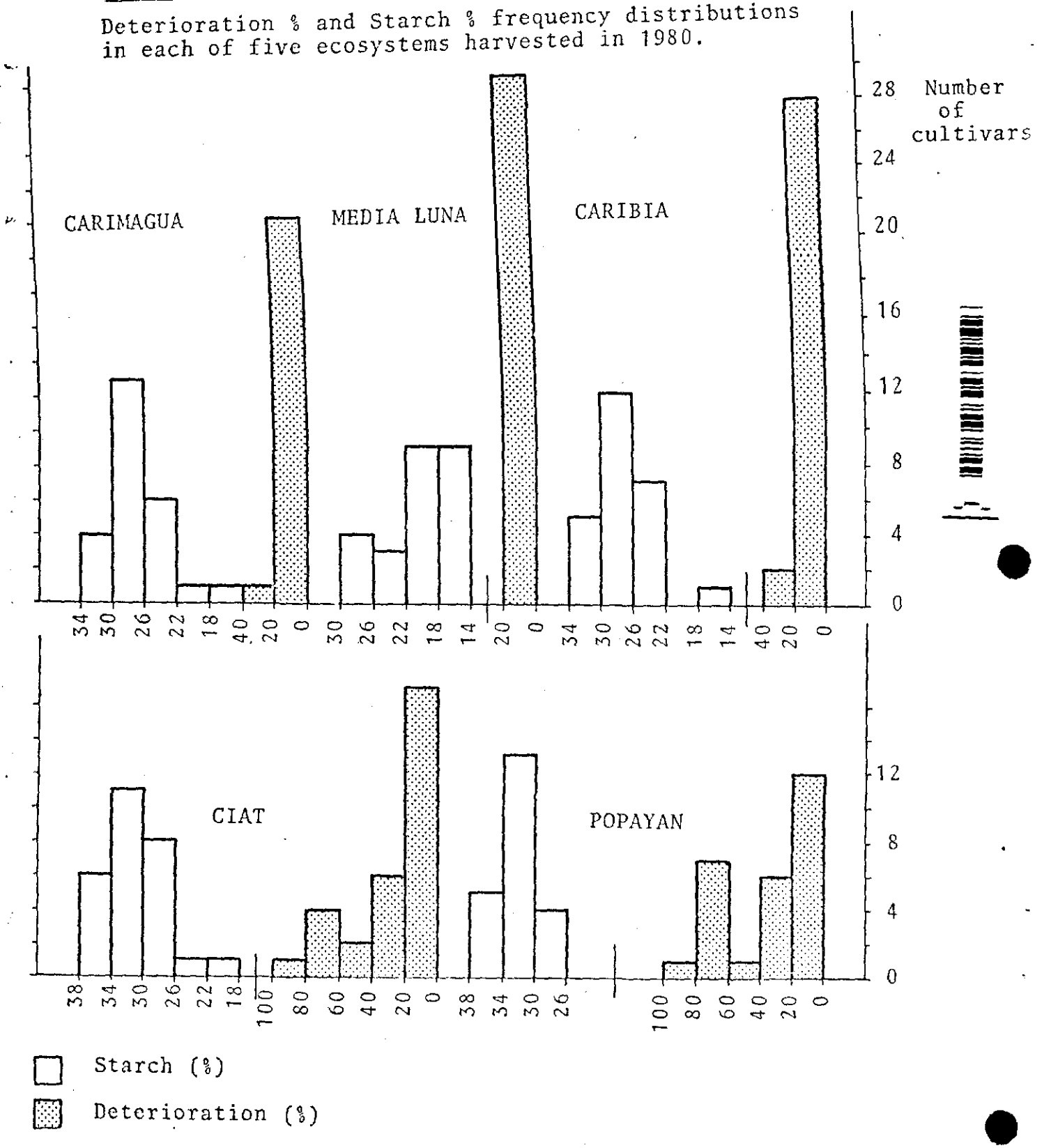


Table 2. STARCH AND DETERIORATION CORRELATIONS IN EACH ECOSYSTEM

	mean Deterioration (%)	mean Starch (%)	Correlation coefficient	level of significance
CIAT	26.68	30.91	0.680	0.1%
POPAYAN	27.02	28.27	0.558	1 %
CARIBIA	4.27	26.82	0.371	10 %
MEDIA LUNA	1.62	20.55	0.341	n.s.
CARIMAGUA	1.45	26.67	0.264	n.s.

Table 3.

Deterioration and other characters of 10 cultivars harvested in Popayán Sept.-1980

CULTIVAR	Deterioration (%)	Starch (%)	Defoliation (%)	Production (ton ha^{-1})	Foliage wt $plant^{-1}$ (kg)
Regional Negrita	76.01	29.48	45	20.87	0.755
Regional amarilla	77.85	33.98	35	23.80	1.44
CMC 92	78.85	30.93	35	26.27	1.50
Sata Dovio	65.78	31.31	25	27.30	2.256
CMC 39	66.10	33.60	76	17.86	0.647
M Col 113	59.35	28.41	53	15.82	0.768
CMC 40	41.74	28.41	79	3.10	0.358
M Mex 59	6.00	25.74	85	0.57	0.27
M Col 1684	3.00	27.34	97	0.93	0.17
M Col 22	(0.00)	20.86	98	0.06	0.09
Correlation with % Deterioration		0.832	-0.874	0.927	0.729
significance		1%	0.1%	0.1%	5%

Table 4. Deterioration (number of vessels cm^{-2}) in fresh tissue placed in contact with deteriorated and fresh (control) tissue after 24hrs at 25°C.

CULTIVAR	CONTROL TISSUE	DETERIORATED TISSUE	REPLICATIONS
M Col 22	1.07	11.57	16
M Col 22	2.00	23.48	16
M Ven 77	3.00	45.20	8
CM 323-375	0.00	36.50	8

Fig.6 Changes in deterioration(%) through time in three cultivars harvested in Popayán in 1980 associated with other harvest parameters.

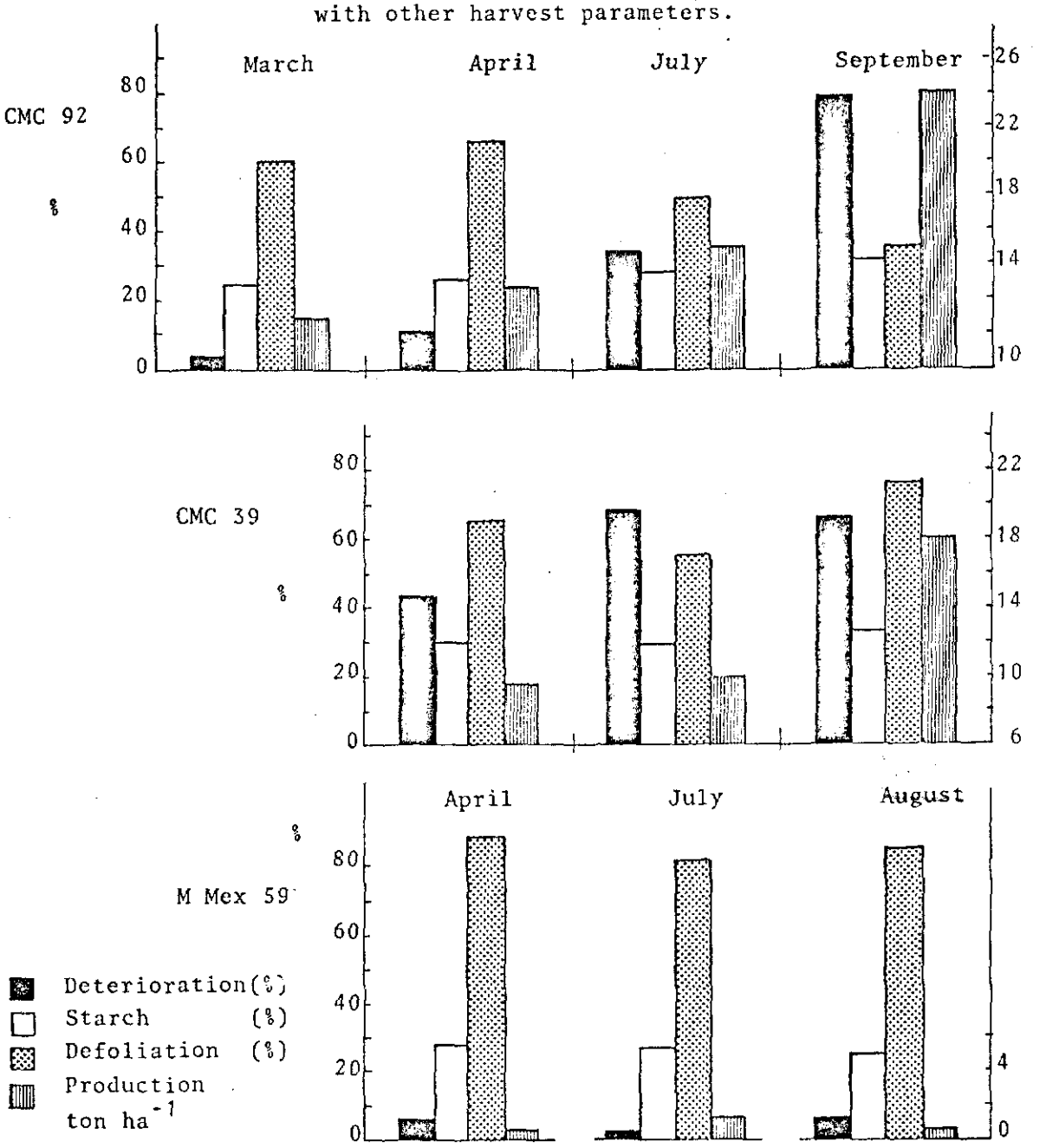
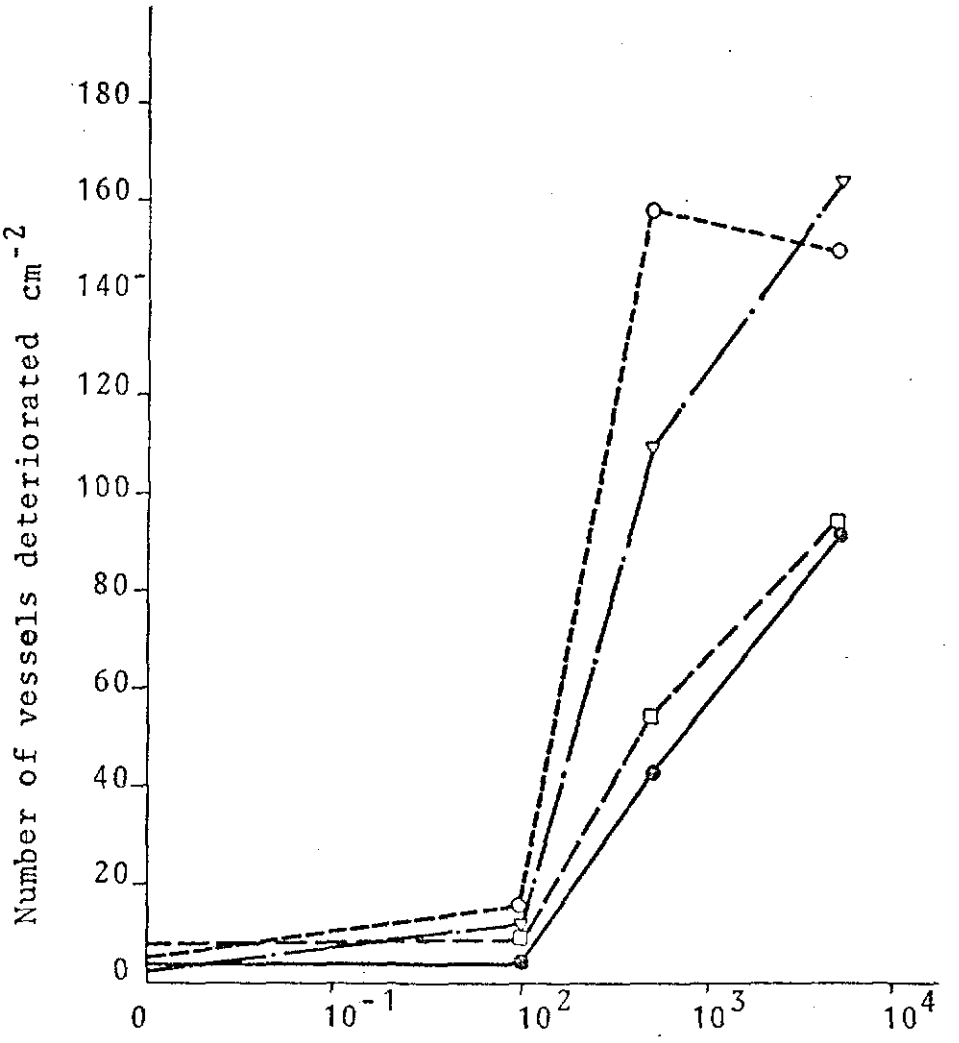


Table 5. Deterioration (number of vessels cm^{-2}) in fresh tissue separated from deteriorated or fresh tissue by a slice of 5% noble agar.after 24hrs at 25°C.

Thickness of Agar	Control Tissue	Deteriorated Tissue	Replications
02	12.20	22.64	25
0.5	3.88	14.76	25
0.2	7.00	34.43	25

Fig.7

Vascular deterioration in root tissue of 4 cultivars of cassava at various concentrations of scopoletin.



——— M Col 1816 Deterioration(%)= 23.07

– – – – M Col 1684 " " = 6.09

—·—·— M Col 22 " " = 18.43

------ M Col 113 " " = 14.86

Fig.8

The effect on deterioration of applications of scopoletin (5mg ml^{-1}) to pruned and unpruned root tissue of M Col 22.

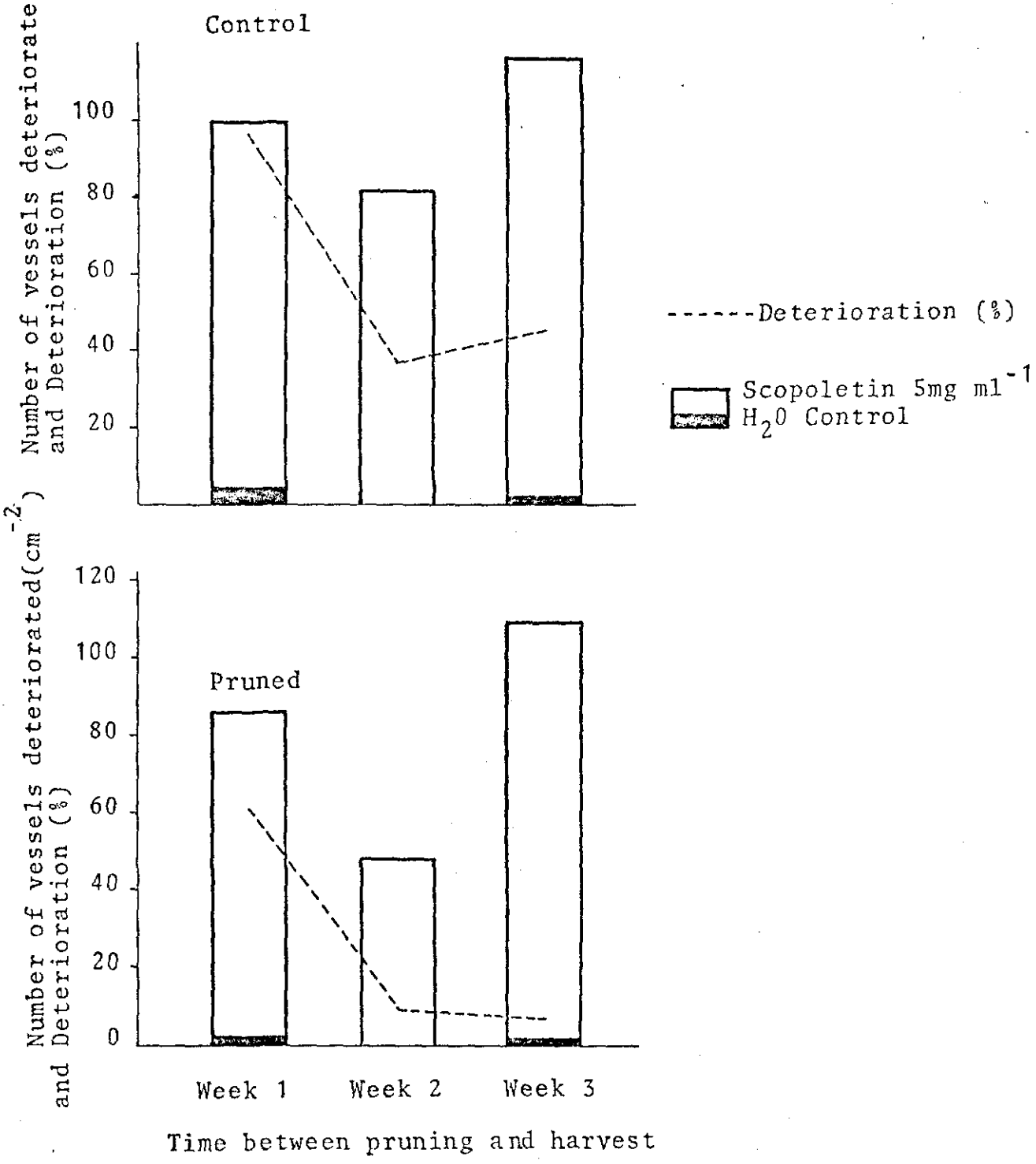


Fig.9

The response of tissue resistant to physiological deterioration from stored (cured) roots to applications of scopoletin.

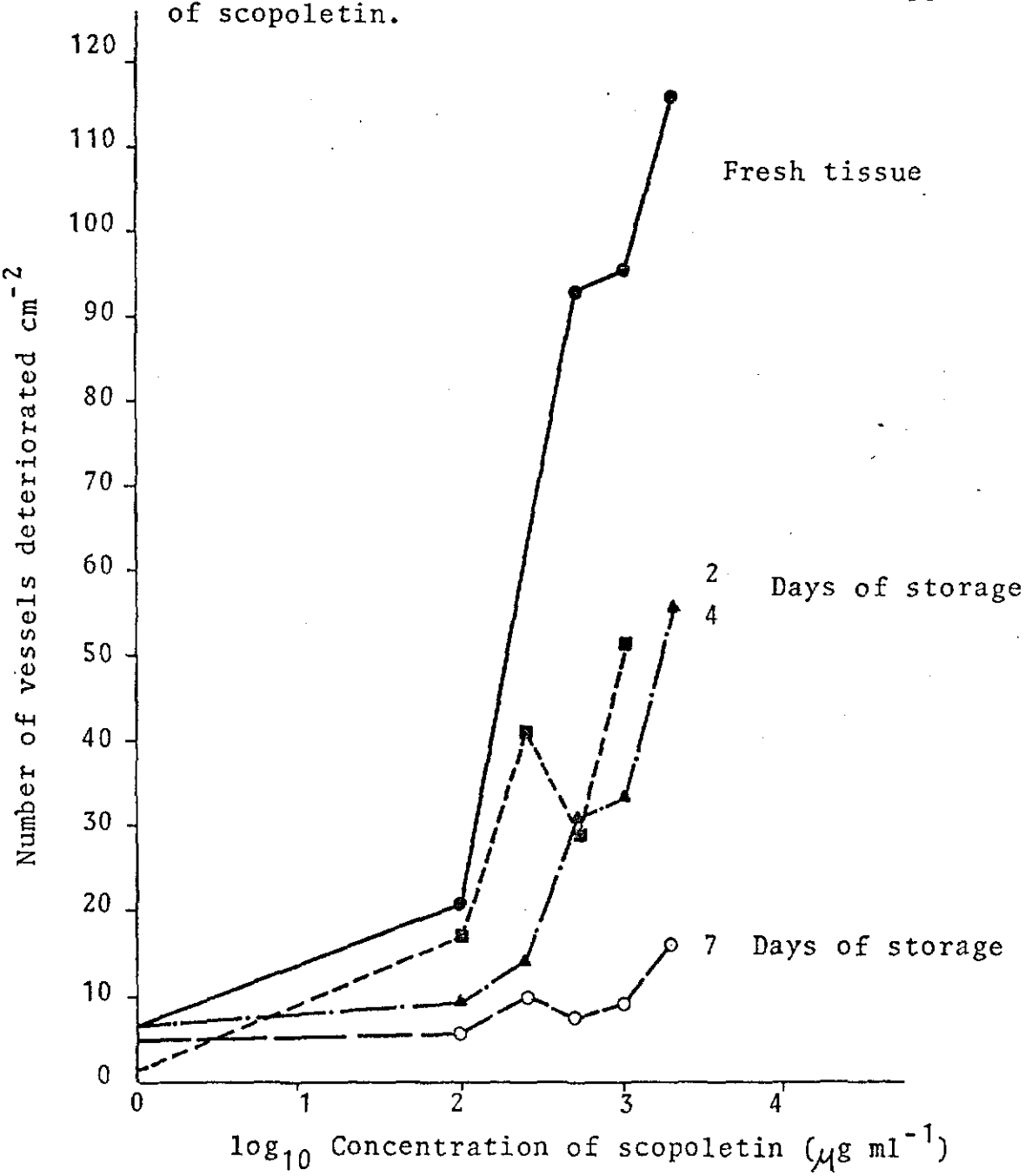


Fig.10 Cycloheximide effects on deterioration in cubes of root tissue.