Effect of virus-stabilizing additives on protein extraction and mechanical transmission of unstable viruses

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The infectivity of leaf sap from virus diseased fruit trees is often low and is lost quickly. It can be increased by the addition of stabilizing substances or by separation of the virus from plant materials [1]. As was pointed out by others [2-4] and shown in our own investigations, the stabilization effect is connected with improved extraction of protein. There is a good correlation between extractibility of protein and mechanical transmissibility of viruses to herbaceous hosts.

Proteins were extracted from leaves of apple, pear, sour cherry and peach by addition of 4 parts (v:w) of distilled water (I), 0.067 M phosphate buffer pH 8.0 (II), and a mixture of equal parts of 0.015 M sodium diethyldithiocarbamate, 0.001 M N, N′-diphenylthiourea, and 0.03 M caffeine in 0.067 M phosphate buffer pH 8.0 (III). Soluble protein of the centrifuged crude extracts (15 min, 15.000 g) was determined by the Kjeldahl method after precipitation with a final concentration of 5% (w:v) trichloroacetic acid. The amount of total protein was calculated by nitrogen determination of the sediment after extraction of crushed plant material with 5% trichloroacetic acid.

The mechanical transmission of fruit tree viruses, especially from pome fruits is very difficult in summer months (Table 1), whereas it is easy to transmit viruses from peach leaves during the whole vegetation period. We found that these are correlated with the protein extractibility. In summer months we seldom extracted more than 1% of total protein from apple leaves (Table 2), whereas it is possible to extract 27.7% protein from peach leaves with phosphate buffer. The relative good results of transmission from forced branches in early spring correspond to higher protein values (16.4) especially when we used virus-stabilizing additives. There was a close coincidence of protein extraction and suitability of leaves and extractants for mechanical virus transmission (Table 2). The same connection could be established by comparing apple leaves and petals (Table 3). Petals were more suitable for protein extraction and virus transmission than leaves.

During protein extraction with buffers of pH 8.0 the pH-value is going down till 5.4 by the high buffer capacity of the acid cell saps. In many instances this decrease is the cause of poor protein extraction and virus transmission. Moreover the phos-

Table 2

Protein extracted from leaves of different fruit tree species (in per cent of total protein)

Extractant	Date	Apple	Pear	Sour cherry	Peach
I	16.4	0.4	0.39	6.8	32.2
II		6.3	20.4	12.4	47.1
III		11.5	21.6	22.6	51.1
I	20.5	0.28	0.15	0.9	21.2
II		1.2	6.6	1.3	22.8
III		0.55	7.9	2.3	28.6
I	28.6	0.12	1.8	0.43	6.5
II		0.58	6.1	1.0	27.7
III		0.35	6.3	0.65	26.8

phate buffer seems to be unsuited for protein extraction from apple leaves. Neither the readjusting of the decreased pH to a final value of 8.0 by diluted NaOH nor an increase of the phosphate buffer capacity by higher ionic strength led to a better extraction of protein.

Table 3

Protein extracted from leaves and petals of "Spy 227" apple (in per cent of total protein) 18.5.1967

Extractant	Leaves	Petals
I	0.58	13.7
II	0.35	31.4
III	0.12	38.9

The unfavourable effect of the phosphate buffer on protein extraction especially of apple leaves (Table 2) can be compensated for by addition of nicotine sulphate. 2% nicotine sulphate in phosphate buffer pH 8.0 increased the protein extraction of apple leaves from 1.2 to 39.2% maximum (Table 4). This is more than with

Table 4

Effect of nicotine sulphate on protein extraction from leaves of apple "Spy 227" in per cent of total protein (Aug./Sept. 1967)

	0.067 M phosphate buffer pH 8.0	0.6% nicotine sulphate in 0.067 M phosphate buffer 8.0	2% nicotine sulphate in 0.067 M phosphate buffer 8.0
Protein extracted	1.2	6.8	39.2
pH	7.4	7.6	8.3

1 g of plant material was crushed in a mortar with 12 ml of the extracting medium.

Table 1

Influence of the season on mechanical transmissibility of fruit tree viruses from leaves of different fruit tree species to herbaceous hosts (1960-1967)

Months and decades		Fruit tree species, viruses, and extractants																					
		apple			pear						sour cherry			cherry								peach								
		CLSV			CLSV			TMV			NRV			NRV			CNRV			CRV			LRV			NRV			CNRV			CRV		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
II	1										—	—		—									+						+	+				
	2														+			+			+													
	3			—											+											—								
III	1					—								+	+		+	+			+													
	2											+				+			+															
	3													+	+			+		+	+			+									+	+	
IV	1																				+			+											
	2									+		+			+				+			+			+			+					+		
	3						+					+			+			+			+													
V	1		+	+		+	+			+		+	+		+			+			+			+			+			+	+			
	2			—			—			—		+			+	+		+						+											
	3			—								—			+	+			—			—			+				+			+		+	
VI	1			—							—	—												—			+	+	+						
	2			—											—												+	+		+					
	3															—			—			—	—					+			+				
VII	1			—											—						—								+						
	2			—																									+						
	3																											+		+			+		
VIII	1																																		
	2																											+		+			+		
	3																									+	+		+						
IX	1															—						—		—			+								
	2																										+		+						
	3			—												—																			
X	1			—																															
	2																																		
	3																																		

Viruses: CLSV = apple chlorotic leaf spot virus; TMV = tobacco mosaic virus; NRV = cherry necrotic ringspot virus; CNRV = cherry chlorotic-necrotic ringspot virus; CRV = cherry chlorotic ringspot virus (Prune dwarf virus); LRV = cherry leaf roll virus.

Extractants: A = 0.067 M phosphate buffer pH 7.0; B = 0.015 M sodium diethyldithiocarbamate in 0.067 M phosphate buffer pH 8.0; C = mixture of equal parts of 0.015 M sodium diethyldithiocarbamate, 0.015 M N, N′ — diphenylthiourea, and 0.03 M caffeine in 0.067 M phosphate buffer pH 8.0.

Sings: + = transmission possible without difficulties; (+) = transmission sometimes possible; — = transmission not possible.

nicotine sulphate in borate buffer (23.6%) or in water (19.6%). The effect of nicotine sulphate probably is due to its alkalinity, compensation of the unfavourable effect of phosphate buffer, and reaction with tannins. Increasing the portion of extractants I and II did not increase the protein contents of leaf extracts significantly. The use of 0.2 M borate pH 8.0 however produced increase ranging from 1.1% (1:4) to 2.8 (1:16) and 17.9% (1:64). Simultaneously the final pH increased from 5.4 to 6.2 and 7.7, respectively. A suitable buffer and an optimal pH seem to be the most important factors for protein extraction and probably for virus transmission from fruit trees, too. These dominant factors are supplemented by stabilizing substances (Table 5).

Table 5

Effect of stabilizing substances on protein extraction from leaves of apple and pear in per cent of total protein (June 1967)

	0.2 M borate buffer pH 8.6	Stabilizing substances from extractant III in 0.2 M borate buffer pH 8.6
Apple	19.0	23.3
Pear	26.9	40.0

The material was crushed in a mortar with 0.2 M borate buffer pH 8.6 (1:16) with and without stabilizing substances and the pH, which went down to 7.8 (apple) and 7.2 (pear) was readjusted to pH 8.0 with diluted NaOH.

The results of protein extraction correspond to mechanical transmissions of pome fruit viruses. Best success in transmission of chlorotic leaf spot virus resulted from the use of petals of apple and pear as inocula. By addition of 5-10 parts of 0.2 M borate buffer pH 9.0 with and without stabilizing substances to one part of leaf material (final pH = 8.0) we have been able to transmit chlorotic leaf spot virus from apple (Lord Lambourne, Malus A2) and pear (Beurre Hardy) as well as a tobacco mosaic virus strain and a probably new virus from pear to *Chenopodium quinoa* Willd. in late July and early August, respectively. Further details will be published in the *Phytopathologische Zeitschrift*.

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