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Comparison of the effects of chlorite-oxidized oxyamylose and polyacrylic acid on the multiplication of phytopathogenic viruses

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Summary

Polyacrylic acid (PAA) and chlorite-oxidized oxyamylose (COAM) inhibit the multiplication of tobacco mosaic virus (TMV) in leaf disks by up to 50%. The reduction in TMV content is time-dependent and decreases with longer time intervals between the virus infection and the application of substances. The multiplication of potato virus X (PVX) in leaf disks is not affected by either PAA or COAM. In intact plants PAA produces a strong antiviral effect on both PVX and red clover mottle virus (RCMV). The effect produced by COAM is much less pronounced, although this substance is less toxic and could be used in a higher concentration than PAA. Neither of these compounds has a significant influence on the development of virus-induced necroses in *Nicotiana glutinosa, Gomphrena globosa* or *Phaseolus vulgaris* plants when administered one day before or after virus infection.

chlorite-oxidized oxyamylose; polyacrylic acid; tobacco mosaic virus; potato virus X; red clover mottle virus

Introduction

PAA and COAM are polycarboxylates showing striking activity against various animal viruses [1]. Compared to PAA whose antizooviral effect has been described by various authors, COAM stands out by its lower level of toxicity. Its antizooviral activity may or may not be mediated by interferon production. The effect of PAA on plant virus infection and on the induction of systemic antiviral resistance has been reviewed by Gianinazzi [3]. The effects of COAM in plant virus systems have, to the best of my knowledge, never been reported.

Materials and Methods

Substances

Polyacrylic acid (Versicol) (M_r 3500), a gift from Dr. White of Rothamsted Experimental Station, U.K., was sprayed as a 0.1% aqueous solution on the upper surface of the host plant. COAM was kindly supplied by Prof. Dr. E. De Clercq (Rega Institute, Katholieke Universiteit Leuven, Belgium). It was applied as a 1% solution. The concentrations of both compounds in culture medium for leaf disks were one-tenth of those used for whole plants (see Table 1).

Viruses

The test viruses, tobacco mosaic virus (TMV, green strain), potato virus X (PVX, H 19), and red clover mottle virus (RCMV, TpM 36), were propagated in their respective systemic hosts.

Host plants

Nicotiana tabacum L. 'Samsun' was used as a systemic host for TMV and PVX, and *Pisum sativum* L. convar. *speciosum* (Dierb.) Alef 'Nadja' served as the host for RCMV. *Nicotiana glutinosa* L., *Gomphrena globosa* L. and *Phaseolus vulgaris* L. 'Perlicka' plants were used as hosts for TMV, PVX and RCMV, respectively.

Plant culture, preparation of leaf disks and application of substances

Plants were cultured in an air-conditioned room (16 h day; 9000 lux; 20 ± 2°C). About 8–10 weeks after seeding, i.e. when the plants were in their four-leaf stage, two fully developed leaves of *N. tabacum* 'Samsun' were inoculated with crude preparation of TMV. In the case of pea plants, 10 days after seeding all leaves were inoculated with crude sap from pea plants infected with RCMV.

Leaf disks of 1 cm diameter were punched out of inoculated leaves immediately after infection as well as 2 and 24 h after infection. The leaf disks were transferred to Vickery's culture solution [8] containing the test compound (for concentration see Table 1) and incubated for 4–5 days. The virus contents given in Table 1 are based on average values obtained from 10 samples of 10 leaf disks each. To establish the influence of PAA and COAM on the systemic spread of viruses, pea and tobacco plants were sprayed three times before and after virus inoculation. The concentration of the compounds is given in Table 2. The virus content values shown in Table 2 are means of about 15 plants. The experiment was repeated twice.

To evaluate the effects of PAA and COAM on a hypersensitive reaction, two young fully developed leaves were chosen from each of the 15 *N. glutinosa* plants, with one half of each leaf being treated with the test solution 1 day before and 1 day after infection. The other half-leaves were used as controls. An analogous procedure was followed with *Phaseolus vulgaris*. In the latter case, however, only the primary leaves were used. For *Gomphrena globosa*, whole leaves were used rather than halves.

30 leaves were tested per experiment. The lesions were recorded 4–5 days after infection. The experiment was repeated twice.

Virus assay

The virus content per 10 leaf disks or one leaf was determined serologically by the precipitin test [6,7], based on the reaction with coat protein, and by a bio-assay based on the infectivity of virus RNA. The virus growth curves are depicted in Figure 1. The virus content in the leaf disks of *N. tabacum* and *P. sativum* was established 5 days after infection. The virus content in systemically infected plants was determined at the following times: the upper of the two tobacco leaves primarily infected with TMV, at 7 days after infection; tobacco leaves with secondary TMV infection, at 18 days after infection; leaves primarily infected with PVX, at 6 days after infection, and leaves secondarily infected with PVX, at two weeks after infection. Each leaf provided one test sample. The secondarily infected tobacco leaf chosen was the next but one above the upper inoculated leaf. RCMV-infected pea plants were harvested at 9 days after infection. In this case each plant provided one sample representing a mixture of primarily and secondarily infected tissue.

The precipitin test performed with pre-clarified sap (centrifugation 10 min, 5600 × *g)* diluted in a geometric manner, and the dilution end point at which precipitation was still visible was determined. The arithmetic mean of these end point dilutions was taken as the average end point dilution (aED). As described by Schuster and Vassilev [9], the difference between the average end point dilution of the control and that of other samples was calculated for virus content, using the formula:

$$\text{Inhibition } (\%) = 100 - \frac{\text{aED}_{\text{variant}}}{\text{aED}_{\text{control}}} \times 100$$

Because of the geometric dilution of virus-containing crude sap, aED represents exponents of 2, and the differences in virus content are calculated from the antilogarithms to 2.

The dilute crude sap was applied, by rubbing, onto the leaves of the hypersensitive host. Here, the concentration was chosen so as to give a direct correlation between virus concentration and the number of necroses.

The significance of the differences between arithmetic mean of the local lesion numbers was determined by Student's *t*-test.

Results and Discussion

As can be seen from the results given in Table 1, COAM and PAA reduced the content of TMV in leaf disks by up to 50%. Moreover, the multiplication of TMV was more decreased if the time between infection and application of active substances was short. In all test systems the virus content was enhanced if the disks are punched out of the leaves not immediately but 2 h after inoculation. With tobacco leaves, the TMV content increased from 4.4 to 5.4 aED and the PVX content increased from 3.6 to 4.8 aED. In contrast with TMV, the multiplication of PVX in leaf disks was not markedly affected. Leaf disks obtained from *Pisum* were heavily damaged when placed in

TABLE 1

Effect of COAM and PAA on virus multiplication in leaf disks

Virus host plant	Time of punching out of leaf disks[a]	Virus content				
		Control aED[b]	COAM (0.1%)		PAA (0.01%)	
			aED[b]	%I[c]	aED[b]	%I[c]
TMV	I	4.4	3.6	+42.6	3.4	+50.0
Nicotiana	II	5.4	4.4	+50.0	4.5	+46.4
tabacum L.	III	5.6	5.4	+12.9	4.9	+29.3
'Samsun'						
PVX	I	3.6	3.8	-12.9	3.8	-12.9
Nicotiana	II	4.8	4.6	+12.9	4.4	+24.2
tabacum L.	III	4.6	4.2	+24.2	4.2	+24.2
'Samsun'						
RCMV	I	3.4	Leaf disks damaged			
Pisum sativum L.	II	5.2	(no virus evident)			
convar. *speciosum*	III	5.0				
(Dierb.) Alef						
'Nadja'						

[a] I, immediately after infection; II, 2 h after infection; III, 24 h after infection.
[b] aED = average end point dilution.
[c] %I = inhibition compared with control.

nutrient solutions containing COAM or PAA, the damage manifesting itself in the decomposition of chlorophyll and in reduced tissue turgor. No virus was detected in such damaged leaf disks.

At the concentrations tested, COAM and PAA had no visible effects on the morphology of plants over a period of 18 days. From Table 2 and Figure 1 it is clear that TMV multiplication was not reduced in the intact host plants after application of the substances. The PVX content is influenced by COAM to a small degree only. But contrary to COAM and to the leaf disks system, PAA inhibited the multiplication of PVX in whole plants.

In leaves secondarily infected with PVX, about 80% inhibition was observed by the serological method, and about 40% inhibition was found when using the bioassay (Table 2). From other experiments we know that differences between the serological virus detection method and bioassay [4] may indicate differences in the effect on virus protein or RNA synthesis [6]. PAA injected in *N. tabacum* 'Xanthi-nc' reduced PVX multiplication only slightly [4]. However, our results with *N. tabacum* 'Samsun', obtained by a changed modus of treatment, indicate that PAA drastically limits the multiplication or spread of PVX. The RCMV titer in pea plants was reduced by about 70% and 80% following COAM and PAA treatment, respectively.

These results reveal that both PAA and COAM have antiphytoviral activity, first for TMV multiplication in leaf disks and RCMV multiplication in pea plants, but the

TABLE 2

Effect of COAM and PAA on the multiplication of viruses in systemically infected host plants, detected in harvested leaves (for details see Materials and Methods 'virus assay')

Virus host plant	Plant material	Virus content									
		Control		COAM (1%)				PAA (0.1%)			
		aED[a]	LL[c] per leaf	aED[a]	%I[b]	LL per leaf[c]	%I[b]	aED[a]	%I[b]	LL per leaf[c]	%I[b]
TMV *Nicotiana tabacum* L. 'Samsun'	Primarily infected leaves	6.8	54 ± 12	7.2	-24.2	62 ± 12	-14.8	6.6	+12.9	48 ± 11	+11.2
	Secondarily infected leaves	8.1	64 ± 14	7.4	+38.4	58 ± 7	+ 9.4	7.8	+18.8	56 ± 8	+12.5
PVX *Nicotiana tabacum* L. 'Samsun'	Primarily infected leaves	4.6	32 ± 8	5.3	-38.4	38 ± 9	-18.8	3.6	+50.0	20 ± 8	+37.5
	Secondarily infected leaves	4.7	28 ± 6	5.2	-29.3	35 ± 7	-25.0	2.2	+82.3	16 ± 4*	+42.8
RCMV *Pisum sativum* L. convar. *speciosum* (Dierb.) Alef 'Nadja'	Whole plants	5.5	-	3.6	+73.2	-		2.9	+83.5	-	

[a] aED = average end point dilution.
[b] %I = inhibition compared with control (%).
[c] LL = local lesions.
* Only significant difference in lesion numbers, compared with control ($P < 0.05$).

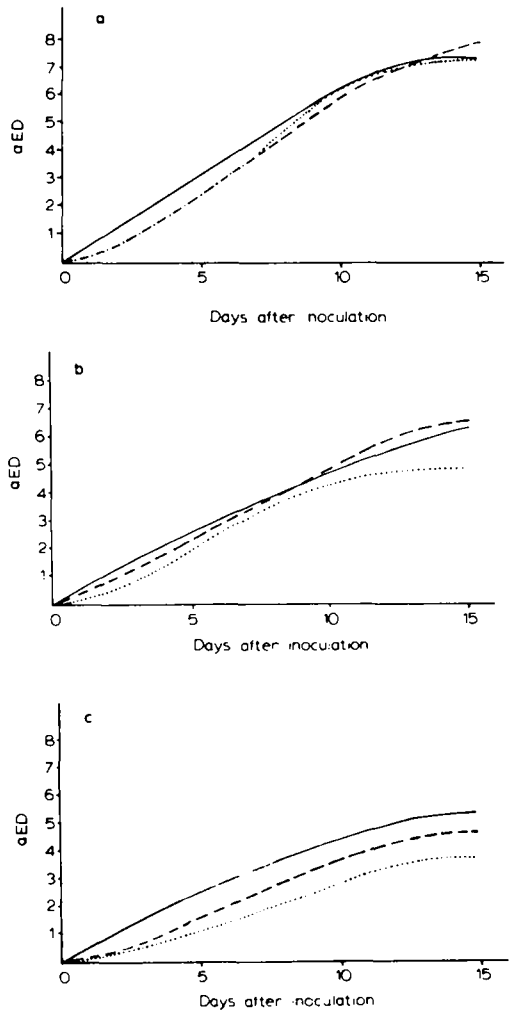

Fig. 1. Growth curves of TMV (a), PVX (b), and RCMV (c). Systemic host plants were inoculated, and 30 leaf disks were punched out of the inoculated (primarily infected) leaves at different times after inoculation. The plants were treated as described under Materials and Methods. The virus content was determined by the precipitin test. aED = average end point dilution. ——, virus-infected, untreated plants (control); ---, virus-infected plants treated with COAM; ·····, virus-infected plants treated with PAA.

effect of COAM is less pronounced than that of PAA. According to previous investigations, PAA is capable of inducing complete resistance, in *N. tabacum* 'Xanthi-nc', to infections with TMV and tobacco necrosis virus [3] and is also capable of suppressing systemic virus infections [4,5]. COAM has not yet been tested for its effects on plant viruses. As originally demonstrated in animals [1], COAM is far less toxic to plants than PAA. Whilst 1% solutions of COAM are tolerated by plants without any untoward effects, 1% solutions of PAA kill the leaves. Regarding the inhibition of

virus synthesis, there are virus-specific differences in the inhibitory effects of PAA and COAM. The following order has been obtained for the inhibition of virus multiplication: TMV < PVX < RCMV.

Finally, attempts were made to determine the effects of PAA and COAM on the formation of necrotic lesions. The number of local lesions was not reduced significantly by either of the two compounds tested (Table 3).

The situation is not comparable with the reported PAA induction of complete resistance to TMV in *N. tabacum* 'Xanthi-nc'. This may be due mainly to the fact that in our experiments we used *N. glutinosa* and PAA was administered one day before and one day after virus inoculation. To induce complete resistance to TMV, however, PAA injection into tobacco leaves should be made two or three days before inoculation [3]. Another point may be the mode of application. Unlike other workers, we sprayed the solution onto the surface of leaves, i.e. uptake by epidermis cells is necessary. Differences in metabolic processes may also play a role because several observations suggest that the effect of PAA is correlated with changes in the protein metabolism of host plants. New host proteins are synthesized, both in association with hypersensitive reaction and after PAA application [3].

The results obtained with PAA and COAM raise the important question as to the mode of action of these compounds in the partial inhibition of viral synthesis in plants. PAA, COAM and other polycarboxylates such as pyran copolymer are well-known for their interferon inducing properties [9], but this does not mean that their antiviral activity is in fact due to interferon production. Although the resistance-inducing effect on plant viruses is probably connected with the synthesis of interferon-like substances and the pathogenesis is connected with the production of b-proteins [3], there are no

TABLE 3

Effect of COAM and PAA on the formation of necrotic lesions after virus infection

Virus host plant	Number of local lesions per half-leaf		
	Control	After application of	
		COAM (1%)	PAA (0.1%)
TMV *Nicotiana glutinosa* L.	26 ± 8	16 ± 5	21 ± 5
PVX *Gomphrena globosa* L	47 ± 8*	47 ± 11*	40 ± 5*
RCMV *Phaseolus vulgaris* L. 'Perlička'	29 ± 6	25 ± 5	22 ± 7

* Per leaf.

indications of the production of such substances as to the drug-induced inhibition of virus multiplication in systemically infected hosts. The results obtained with systemically infected leaf disks show that a viral infection may be inhibited especially in those cases where there is but little time between virus inoculation and drug application. This relation would not apply to hypersensitive hosts.

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