In order to confirm the previous result, a second test was performed. Survival on *Opuntia* medium of adults from population P1 was compared to that of adults from an experimental population (C2) with an identical origin but kept on standard *Drosophila* medium instead of on *Opuntia* rots (see Ruiz[6] for details). Four vials (with 10 individuals each) per sex and population were established. As in the previous case, adults to be tested had been reared under near optimal conditions on standard *Drosophila* medium. Adults were transferred to a new vial with fresh substrate every five days. The number of survivors was registered daily.

Figure 2 shows the results of this test: Adults from population P1 lived longer than those from population C2. On the other hand, in both cases females lived longer than males. A two-way analysis of variance performed with the number of survivors at day 10 yielded the following result: F(populations) = 32.53, $p < 0.001$, and F(sexes) = 10.24, $p < 0.01$. Analogously, for the data of day 20: F(populations) = 84.76, $p < 0.001$, and F(sexes) = 9.42, $p < 0.01$. Approximate times of 50% survival are 7.8 days (C2 males), 10.7 (C2 females), 14.6 (P1 males) and 19.6 (P1 females).

Three main conclusions can be drawn from the results reported here.

1) *D. buzzatii* adults are able to utilize *Opuntia* rots as feeding substrate while *D. serido* adults are not. The physiological basis of this difference is at present unknown but several possibilities exist. The simplest one, although not the most probable, is that *D. serido* adults cannot metabolize the monosaccharides present in the cladodes of *O. ficus-indica*, i.e. glucose, fructose and galactose[9]. An alternative hypothesis is the presence in the *Opuntia* medium of toxic compounds, such as alkaloids which have been detected in small amounts in *Opuntia* cladodes[9]. Toxic compounds can be also produced by microorganisms associated with the rotting process. Several volatile compounds, such as ethanol, methanol, ethyl acetate, isobutanol and isoamyl alcohol have been identified as cactophilic yeast metabolites[10]. In addition, 2-propanol, n-propanol and acetone have been found by us in significant amounts in natural rots of *O. ficus-indica* from southern Spain. All these chemicals can occur in our *Opuntia* vials and may be potentially toxic to *D. serido* adults. Preliminary studies (F. Peris, personal communication) show that the egg to adult viability of *D. serido* in *Opuntia* rots is comparable to that of *D. buzzatii*. Moreover, *D. serido* adults reared from this substrate in the laboratory are fertile and reproduce normally when transferred to culture bottles with standard *Drosophila* medium. However, if placed on *Opuntia* medium, they die before maturity and this explains the extinction of the *D. serido* population referred to above. All these observations lead to the idea that adult selection could be the crucial step in the niche differentiation between *D. buzzatii* and *D. serido*.

2) The mortality observed in populations P1 and P2 was selective and resulted in an improved adult survival on the *Opuntia* medium. This means that intrapopulation variability does exist for the character and that adaptation to feed on this substrate may arise through natural selection. Whether this kind of selection is actually taking place in cactiphilic *Drosophila* species in their natural habitats in unknown.

3) *D. buzzatii* adults lived longer on the *Opuntia* medium when transferred periodically to new tubes with fresh substrate. This suggests that the rotting process makes *Opuntia* tissues unsuitable for adult feeding. Fellows and Heed[5], trying to explain the observation cited above, suggested that the presence of *Drosophila* larvae was probably the cause of the inability of cactus tissues to support adult life. Our results, however, show that even in the absence of larvae, *D. buzzatii* adults do not readily survive on *Opuntia* medium. Depletion of sugar or the accumulation of toxic compounds produced by the microorganisms growing on the medium might be the cause.

1 This work has been supported by the Comisión Asesora de investigación Científica y Técnica, Spain, by funds to project 4514/79 awarded to the third author.

2 Van Herrewege, J., C.r. Acad. Sci. Paris (D) *276* (1973) 2565.
3 Sang, J.H., in: The Genetics and Biology of Drosophila, vol. 2a. Eds M. Ashburner and T.R.F. Wright. Academic Press, London 1978.
4 Carson, H.L., Harold L. Lyon Arboretum Lecture *2* (1971).
5 Fellows, D.P., and Heed, W.B., Ecology *53* (1972) 850.
6 Ruiz, A., Ph. D. thesis, University of Santiago, Spain, 1982.
7 Ruiz, A., Fontdevila, A., and Wasserman, M., Genetics *101* (1982) 503.
8 David, J., Bull. Biol. *93* (1959) 472. A modified formula of David's medium was used in our experiments.
9 Kircher, H.W., in: Ecological Genetics and Evolution. Eds W.T. Starmer and J.S.F. Barker. Academic Press, Australia 1982.
10 Fogelman, J.C., in: Ecological Genetics and Evolution. Eds W.T. Starmer and J.S.F. Barker. Academic Press, Australia 1982.

0014-4754/85/010129-03$1.50 + 0.20/0

Inhibitory effect of some heteropolyanions on potato virus X[1]

B. Sharma, K.D.S. Yadav, S.N. Ram and A.K.S. Baghel

Departments of Chemistry and Botany, University of Gorakhpur, Gorakhpur 273001 (India), 21 July 1983

Summary. Heteropolyanions like 12-tungstozincic acid (i.i.), potassium 13-vanadomanganate (i.v.), potassium 13-vanadonickelate (i.v.) and sodium tungstoborate produced an inhibitory effect on potato virus X. Amongst these, tungstozincic acid was found to be the most potent.

Key words. Potato virus X; heteropolyanions; 12-tungstozincic acid; potassium 13-vanadomanganate; potassium 13-vanadonickelate; sodium tungstoborate; antiviral activity.

Biological roles of heteropolyanions in modifying the cell membrane and affecting the adsorption and penetration of viruses are well known[2–5]. The antiviral activity of silicotungstate, and its in vitro inhibitory action on murine leukemia, sarcoma virus[5,6] and other non-neogenic viruses have earlier been reported[7–9]. Silicotungstate has been found to inhibit Escherichia coli DNA and RNA polymerases extracted from mouse $3T_3$ cells[10]. The heteropolyanion tungsto 2-antimonate protected mice against Friend and plasma variant induced leukemias[5]. It had an antiviral effect and its action did not require direct preliminary contact between virus and product. The present report deals with the effect of 4 heteropolyanions, 12-

Effect of heteropolyanions on the infectivity of potato virus X using test plant *Chenopodium amaranticolor*

Chemical species	Dilution (μg/ml)	Average No. of local lesion/leaf* Treated	Control	Percent decrease in virus activity	Incubation period (days)
12-Tungstozincic acid	1000	1	75	99[a]	7
	100	7	72	90[a]	7
	1	12	64	81[a]	8
Potassium 13-vanadomanganate	1000	9	56	84[a]	6
	100	32	83	61[a]	6
	1	44	80	45[a]	8
Potassium 13-vanadonickelate	1000	28	71	61[a]	7
	100	38	68	44[a]	7
	1	46	59	22[b]	8
Sodium tungstoborate	1000	55	86	36[b]	6
	100	65	82	21[b]	7
	1	64	72	11	8
Sodium tungstate	1000	51	63	19	7
	100	72	80	10	8
	1	78	78	0	7
Sodium silicate	1000	49	58	16	6
	100	55	62	11	8
	1	67	68	1	7

Differences due to treatment with heteropolyanion are significant at 1% level (a) and at 5% level (b). *Average number of local lesions/leaf per treatment are taken from 10 leaves.

tungstozincic acid, potassium 13-vanadomanganate, potassium 13-vanadonickelate and sodium tungstoborate on potato virus x (PVX).

Material and methods. The heteropolyanions were prepared by the well-known methods described earlier[11-14] and their purity was checked by IR study. A pure culture of potato virus X was maintained on tobacco (*Nicotiana tabacum*, var. white burley). *Chenopodium amaranticolor*, a local lesion host of the virus was employed as test plant for the assay of the virus. Plants of the same age, height and vigor were used in all treatments, after leaving the youngest leaves close to the apex. The local lesion method was used for virus assay. Whole leaves were used for various treatments and control was separately maintained.

For the preparation of an inoculum of the viruses, young infected leaves showing severe symptoms were macerated with double distilled water (1 ml/1 g of leaf material) in a sterilized mortar. The extract was filtered through 2 folds of muslin cloth, and centrifuged at 5000 rpm for 10 min. The sediment was discarded and the clear supernatant fluid was suitably diluted with double distilled water and used as the inoculum.

Stock solutions of 1 mg/ml of the heteropolyanions were prepared in twice-distilled water and were further diluted as and when needed. Leaf extract (inoculum) from an infected tobacco plant was mixed with different dilutions of heteropolyanions in equal proportions, left for 10 min and then used as virus inoculum. Healthy and vigorously growing plants of *Chenopodium* at the 4-leaf stage were used as test plant.

Inoculations were made gently and uniformly with a forefinger wet with inoculum on the upper surface of the leaves in one direction along both side of the midrib from their base to the top. The leaves were previously dusted with carborandum powder (600 mesh). After inoculation, the leaves were washed with distilled water. The amount of inoculum and abrasive applied were kept constant in each set of experiment. 1 ml of the infective sap mixed with 1 ml of water served as control. All the experiments were carried out in an insect-free glasshouse under natural light conditions.

The data were analyzed statistically[15] by the test of comparison between the control and the individual treatment (check versus treatment) for the significance of the activity of heteropolyanions. Percent inhibition was calculated by the formula $\frac{(C-T) \times 100}{C}$ where C is the number of lesions on control leaves and T is the number of lesions on the treated leaves.

The results are summarized in the table, which demonstrates clearly that 12-tungstozincic acid shows maximum inhibitory activity followed by potassium 13-vanadomanganate, potassium 13-vanadonickelate and sodium tungstoborate. The inhibitory activities of sodium tungstate and sodium silicate are insignificant. The results obtained with tungstozincic acid are very interesting in that it is a transition metal compound of the class (1:12) of heteropolyanions.

1 The authors are thankful to Prof. R.P. Rastogi, Head of the Chemistry Department, University of Gorakhpur for providing necessary facilities. The financial support by Council of Science and Technology, U.P., Lucknow is gratefully acknowledged.

2 Regelson, W., Proc. of Int. Symp. on Reticuloendothelial Syst. and Arterioscleroses. Lake Como, Italy 1966.
3 Solomon, J.J., Glatt, K.K., and Okazaki, W., J. Bact. *92* (1966) 1855.
4 Vaheri, A., Acta path. microbiol. scand., suppl. *171* (1964).
5 Jasmin, C., Chermann, J.C., Herve, G., Tézé, A., Souchay, P., Boy-Loustau, C., Raybaud, N., Sinoussi, F., and Raynaud, M., J. natn Cancer Inst. *53* (1974) 469.
6 Raynaud, M., Chermann, J.C., Plata, F., Jasmin, C., and Mathe, G., C.r. Acad. Sci. Paris, D *272* (1971) 347.
7 Bonissol, C., Kona, P., Chermann, J.C., Jasmin, C., and Raynaud, M., C.r. Acad. Sci. D., Paris *274* (1972) 3030.
8 Jasmin, C., Raybaud, N., Chermann, J.C., Sinoussi, F., and Werner, G.H., Biomedicine *18* (1973) 319.
9 Raybaud, N., Jasmin, C., and Huppert, J., Eur. J. clin. Biol. Res. *17* (1972) 295.
10 Haapala, D., Jasmin, C., Sinoussi, F., and Chermann, J.C., Biomedicine *19* (1973) 7.
11 Brown, D.H., and Mair, J.A., J. chem. Soc. *1958*, 2597.
12 Parshall, G.W., Inorganic syntheses, vol. 15, p. 103. McGraw Hill Book Company, New York 1974.
13 Flynn, C.M. Jr, and Pope, M.T., J. Am. chem. Soc. *92* (1970) 85.
14 Brauer, G., Handbook of Preparative Inorganic Chemistry, p. 1700. Academic Press, New York 1965.
15 Snedecor, G.W., Statistical Methods Allied Pacific (P) Ltd., Bombay 1961.

0014-4754/85/010131-02$1.50 + 0.20/0