Preservation of Lettuce Necrotic Yellows and Some Other Plant Viruses by Dehydration with Silica Gel

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Lettuce necrotic yellows virus (LNYV) is very unstable in vitro (Crowley, 1967) and is therefore difficult to store, even by dehydrating virus-infected leaf tissue, a technique which has been particularly successful for the long-term storage of many plant viruses (McKinney & Silber, 1968). Hunt, Gourevitch & Lein (1958) and Perkins (1962) described ways of preserving microbial stock cultures by dehydration with anhydrous silica gel, and these methods have been applied to a range of microorganisms (see Grivell & Jackson, 1969).

In this paper we report that LNYV has been preserved for several years by the technique described by Perkins (1962) for storing fungi, in which a suspension of the fungal spores is mixed with skim-milk and then with anhydrous granular silica gel. Tomato spotted wilt (TSWV), cucumber mosaic (CMV) and cauliflower mosaic (CIMV) viruses have also been stored by this method and later recovered.

The S3 strain of LNYV (Stubbs & Grogan, 1963) was propagated in plants of *Nicotiana glutinosa* L. Young systemically infected leaves were homogenized in a mortar and pestle with 0.2M-Na₂HPO₄ (1.0 to 1.5 ml/g, wet-weight of tissue) at around 2°C. The homogenate was centrifuged at 1000g for 5 min. and samples of the supernatant fluid, or of a virus preparation purified as described by McLean & Francki (1967), were mixed with equal volumes of either water or 15% (w/v) reconstituted skim-milk ('Bonlac' obtained from 'Trufood', Glenormiston, Victoria, New South Wales). 'Pyrex' glass tubes (13 x 100 mm.) were half-filled with granular silica gel (previously dehydrated at 175°C for 1½ hr; grade and source as specified by Perkins, 1962). The tubes were stood in crushed ice and samples of virus-in-milk mixtures (0.2 to 0.4 ml.) were added drop by drop to each. The tubes were kept in ice for a further 10 to 15 min., then for a week at 2 to 4°C in a desiccator containing freshly activated silica gel. After a viability test, each tube was sealed with 'Parafilm' and stored over self-indicating silica gel in a sealed jar at 2 to 4°C.

For viability tests, about one-fifth of the gel from a tube was stirred with 1 ml. of either chilled 0.02M-phosphate buffer (pH 7.5) or 0.05M-phosphate buffer (pH 6.5), and the infectivity of the fluid was tested by inoculating it on to leaves of *N. glutinosa* dusted with 500 mesh carborundum (Crowley, 1967). The unused gel was resealed.

For the experiments summarized in Fig. 1 and 2, infected plant tissue was extracted in 0.125M-Na₂HPO₄ (1.0 to 1.5 ml/g. wet-weight of tissue) and was mixed with either water or skim-milk (1:1, v/v) and 0.05 ml. samples were dehydrated with 0.5 g. samples of silica gel. The samples were stored at different temperatures for differing periods and then each was shaken with 1 ml. of 0.05M-phosphate buffer (pH 6.5) and tested for infectivity.

TSWV, CMV and CIMV were also dehydrated with silica gel. TSWV was propagated and assayed on *Datura stramonium* L., extracted from leaf tissue in 0.2M-Na₂HPO₄ (1.0 to 1.5 ml/g. wet-weight of tissue) and washed from the gel with 0.02M-phosphate buffer (pH 7.5). CMV was extracted from *Cucumis sativus* L. cv. Polaris in 0.5M-sodium citrate, resuspended from the gel in 0.005M-sodium tetraborate, and its infectivity tested on the same species. CIMV was extracted from, and tested on, *Brassica pekinensis* Rupr., using phosphate buffer (pH 7.5, as above), either 0.2M or 0.02M. Other conditions were as described for LNYV.
Both crude and purified preparations of LNYV mixed with skim-milk and dehydrated were still infective after 30 weeks storage at 2 to 4°C and a dehydrated purified preparation was active after 2½ years. Preparations stored with skim-milk were much more infective than those stored without (Fig. 1, curve A), since under similar conditions very little, if any, infectivity was recovered from tubes of material dehydrated in its absence. LNYV, dehydrated and later rehydrated without skim-milk and then inoculated after adding skim-milk, gave only 14% of the number of local lesions given by material to which skim-milk had been added before dehydration.

![Graph](image-url)

**Fig. 1.** The effect of skim-milk and temperature on the survival of dehydrated LNYV. ▲—▲, relative infectivity of LNYV dehydrated without skim-milk. The value plotted at each time is the ratio of the mean number of lesions produced by a virus preparation dehydrated without skim-milk to the mean number of lesions produced by the same preparation dehydrated in the presence of 15% (w/v) skim-milk. (Both treatments were kept at 2 to 4°C.) ○—○, relative infectivity of LNYV after storage at 24 to 25°C. Each value plotted is the ratio of the mean number of lesions produced by a virus preparation stored at 24 to 25°C to the mean number of lesions produced by the same preparation stored at 2 to 4°C. (Both treatments included 15% (w/v) skim-milk.)

LNYV-containing plant extracts dehydrated with skim-milk soon lost infectivity when stored at temperatures above 4°C. The relative infectivity of material stored for varying periods at 24 to 25°C is shown in Fig. 1. Infective preparations were obtained from material stored at 37°C for 19 hr and 30°C for 11½ days, but not after 12 weeks storage at 30°C. Although storage of dehydrated virus at room temperature is not recommended, these data show that the dehydrated virus can survive for some time at temperatures above 4°C. Thus virus dehydrated with silica gel may be useful for sending cultures by mail.

The amount of LNYV recovered after dehydration was dependent on the concentration of skim-milk, as illustrated in Fig. 2. A mixture of equal volumes of virus suspension and 15% (w/v) skim-milk seems optimal under the conditions we used.

Preliminary tests show that three other plant viruses may be effectively stored using the same method. Thus, at the last testing TSWV was recovered after dehydration for 50 weeks, CMV after 35 weeks, and CIYMV after 40 weeks. The effect of skim-milk differed with these
three viruses; it seemed to have no effect on the storage of CMV, but CIMV dehydrated with skim-milk maintained infectivity better than when without, and TSWV was only recovered when skim-milk was added.

Although this dehydration technique has been tested here with few plant viruses and for short periods only, it was successful even with the very unstable TSWV and LNYV and thus the method may well be of use in the preservation of a much greater range of viruses for extended periods.

![Graph](image)

**Fig. 2.** Influence of the concentration of skim-milk on the survival of dehydrated lettuce necrotic yellows virus (LNYV). Samples were kept at 2 to 4°C and reactivated after 1 week and again 12 weeks later. Each value plotted is the ratio of the mean number of lesions produced by a virus preparation dehydrated with the specified concentration of skim-milk, to the mean number of lesions produced by the same preparation dehydrated with 15% (w/v) skim-milk. (● — ●, stored for 1 week; ▲ — ▲, stored for 12 weeks.)

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REFERENCES


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