

A Virus Disease of *Datura* Spp.

by

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INTRODUCTION

Many malformed *Datura* plants were noticed on a land lying fallow for the summer. The first impression was that this was damage due to hormone sprays e.g. 2-4D, as these symptoms were similar to those caused by weedkiller preparations.

The plants were not noticeably stunted; but the leaves were severely distorted with exaggerated indentations resulting in sharply serrated margins. They had irregular chlorotic areas and very prominent raised veins. There was an almost complete suppression of the spines on the seed capsules.

After severe frosts, only the skeletons of the plants remained and a count of the spineless pods was possible. Twelve to 15 per cent of the plants were affected.

Preliminary sap transmissions to a few Solanaceous test plants resulted in unmistakable virus symptoms on *Datura*, *Nicotiana glutinosa*, tobacco and petunia, and the causal virus was therefore investigated.

PROCEDURE

Malformed *Datura* plants were collected at intervals throughout the year from different parts of the land. Seed from affected plants was harvested for seed transmission tests; and seed from healthy plants was used for experimental test plants in the glasshouse.

These *Daturas* were probably mixed *D. stramonium* x *D. tatula* hybrids.

Standard virus technique was used throughout this investigation.

RESULTS

1. PHYSICAL PROPERTIES

As no local lesion host was found, these values are based on the systemic symptoms induced on *Datura*, *Nicotiana glutinosa*, *N. langsdorffii* and *N. tabacum*. The source of inoculum was the first three named above.

Thermal inactivation point.....	65°-70°C.
Dilution end point.....	5,000-10,000.
Longevity.....	3-4 days.

2. TRANSMISSION

(a) *Mechanical sap inoculation*.—The virus is readily sap transmissible even without the addition of carborundum powder.

(b) *Insect vectors*.—In glasshouse experiments the following were used:—

(i) *Aphids*. At least two species are efficient vectors: *Myzus persicae* (Sulz.)—small pale green aphid found naturally on *Daturas*.

Aphis craccivora Koch—black aphid colonising groundnuts.

(These two were used only as a criterion of whether or not the virus was aphid transmitted).

- (ii) Whitefly. These are often found in the field, under the *Datura* leaves. All results were negative.

Details of aphid transmission experiments: *Datura* was used as the source of inoculum. Five to ten aphids were fed on excised leaves in Petri dishes for 24 hours, and then transferred to the test plants for a further 24 hours. Symptoms developed on the following plants: *Datura* sp., *Nicotiana glutinosa*, *N. langsdorffii*, *N. rustica*, *N. sanderae*, *N. sylvestris*, *N. tabacum* and *Physalis peruviana*.

A feature of the aphid-infected *Daturas* was the extreme malformation and distortion of their leaves. Often they were reduced to only a thickened midrib or possibly a half lamina only 2 mm wide along the entire main vein (PLATE 2). There was a complete suppression of spines on the capsules. This increase in intensity of symptoms was not apparent on other host plants.

(c) *Seed transmission*.—As the virus distorts the flowers and suppresses the spines on the capsules of *Daturas*, the possibility of the virus being transmitted through the seed was investigated. (The *Datura* Quercina virus was transmitted to 79 per cent of the progeny. Blakeslee 1921).

Seed harvested from *D. stramonium* plants individually infected with six of the nine isolates was sown in sterilized soil. Normal *Datura* seed is black and firm, and the capsules are fully packed. In contrast, the seeds from infected pods are small, white or gray, and shrivelled.

Both strains of the virus reduced the percentage of viable seeds per pod; and from plants infected with the severe strain less than half of the seeds germinated. Not a single seedling showed virus symptoms.

The numbers of seeds sown, germinated and infected were recorded:

Severe strain: 402 seeds sown, 171 germinated i.e. 42.5 per cent (3 isolates).

Mild strain: 90 seeds sown, 77 germinated i.e. 85.5 per cent (3 isolates).

3. HOST RANGE EXPERIMENTS

GROUP A

The following plants developed obvious virus symptoms and the virus was readily transmitted back to *Datura*:

SOLANACEAE: *Datura ferox* L., *D. stramonium* L., *Nicotiana clevelandii* A. Gray, *N. glutinosa* L., *N. langsdorffii* Weinm., *N. rustica* L., *N. sanderae* Sander., *N. sylvestris* Speg. & Comes., *N. tabacum* L. vars. Delcrest, Hicks and White Burley, *Petunia hybrida* Hort. ex Vilm. var. Rosy Morn and *Physalis peruviana* L.

GROUP B

Clear virus symptoms developed on 3 hosts but few transmissions back to *Datura* were positive:

SOLANACEAE: *Capsicum annuum* L., *C. frutescens* L. and *Lycopersicon esculentum* Mill. var. Little Marvel.

GROUP C

Apparent virus symptoms developed on several hosts but all sub-inoculations to *Datura* were negative.

AIZOACEAE: *Tetragonia expansa* Thunb.—diffuse chlorotic blotches on the young leaves.

AMARANTHACEAE: *Gomphrena globosa* L.—local necrotic rings.

CHENOPODIACEAE: *Chenopodium amaranticolor* Coste & Reyn.—local white lesions which become necrotic.

COMPOSITAE: *Callistephus chinensis* Nees—raised darker green blisters on some leaves.

CUCURBITACEAE: *Cucumis sativus* L.—local white necrotic lesions.

LEGUMINOSAE: *Crotalaria juncea* L.—local necrotic specks.

Phaseolus lathyroides L.—systemic chlorotic spots. *Vicia faba* L.—local necrotic rings.

GROUP D

Latent carriers i.e. the test plants were symptomless but sub-inoculations to *Datura* were positive.

COMPOSITAE: *Ageratum houstonianum* Mill., *Dahlia pinnata* Cav., *Zinnia elegans* Jacq.

MALVACEAE: *Althaea rosea* Cav.

PRIMULACEAE: *Primula malacoides* Franch.

SCROPHULARIACEAE: *Antirrhinum majus* L.

GROUP E

The following plants were not susceptible to this virus:

BORAGINACEAE: *Anchusa capensis* Thunb.

CARYOPHYLLACEAE: *Dianthus barbatus* L.

CHENOPODIACEAE: *Beta vulgaris* L.

COMPOSITAE: *Arctotis breviscapa* Thunb., *Sonchus oleraceus* L.

CRUCIFERAE: *Alyssum maritimum* Lam.

GRAMINAE: *Zea mays* L.

LABIATAE: *Salvia splendens* Ker-Gawl.

LEGUMINOSAE: *Lupinus albus* L. var. Sweet, *L. luteus* L. var. Sweet, *Medicago sativa* L., *P. vulgaris* L. var. Canadian Wonder, *Pisum sativum* L. var. Greenfeast, *Trifolium subterraneum* L. *Vigna unguiculata* (L.) Walp.

ONAGRACEAE: *Gaura lindheimeri* Engelm. & Gray.

POLEMONIACEAE: *Phlox drummondii* Hook.

RANUNCULACEAE: *Aquilegia* Hort. sp.

VIOLACEAE: *Viola* Hort. sp.

4. REACTION OF SUSCEPTIBLE SPECIES

After several experiments it was evident that there were differences in the symptoms induced on the test plants by the virus isolates from the many naturally infected *Daturas* used. This was a difference in degree of severity and not a different type of symptom. Therefore it was assumed that two strains of the same virus occurred in that area—mild and severe. This variation was not apparent on all host plants, so the 2 strains are described separately only where the difference was significant.

GROUP A

SOLANACEAE

Datura stramonium. There is no local reaction. The severe strain causes a vein-clearing at the base of the young leaves in 14 days. As these leaves mature the main vein becomes arched downwards and the leaf margins are rolled. Later formed leaves have distinct linear chlorotic areas which fuse and cause distortion; and there are also dark green blisters. The teeth of these leaves are sharper than normal.

These symptoms increase in severity on all new growth until the laminae are reduced to narrow bands of tissue along the midrib from which long "teeth" extend. The last leaves formed before flowering may be stringlike. The flowers are twisted and may also be distorted.

The seedpods or capsules are relatively small and completely smooth or have only a few vestigial spines. PLATE 1. There is a marked dark green network on these bare pods. Fewer seeds are formed and a high percentage are infertile.

The mild strain induces similar early symptoms. However, on later growth the indentations are not as deep, there is less malformation of the laminae and the leaves show an irregular chlorotic mottle. The capsules have isolated short spines, or the spines may be confined to one valve only. PLATE 2.

(The symptoms which develop after insect transmission are described in that section).

Datura ferox. With both strains the reaction is similar to, but not as severe as that of *D. stramonium*. The capsules have few short stubby spines.

Nicotiana clevelandii. With both isolates there is no local reaction; nor is there a preliminary veinclearing or chlorotic spotting. After a month the young developing leaves are misshapen and fluted, and this malformation increases on subsequent leaves.

With the severe strains the laminae are reduced to occasional dark green blisters protruding from the side of the distorted stringlike midrib. The whole plant is extremely stunted.

With the mild strain the leaves are broader but more distorted with frequent dark green blisters and the plant is stunted. PLATE 4.

Nicotiana glutinosa. There is no local reaction. In 6–10 days there is a chlorotic network followed by a mottle. As the leaves mature the chlorosis intensifies to form large blotches on an uneven leaf surface.

In addition to the mottle, the severe strain induces short dark green veinbands, and the leaves are elongated.

Nicotiana langsdorffii. The local reaction is slow in appearing. With the severe strain, large chlorotic blotches develop after a month, or the leaves just become flaccid and later collapse and drop. Leaves inoculated with the mild strain develop chlorotic blotches and become flaccid but do not collapse.

The first systemic symptoms of the severe strain are seen in 10–14 days as a vein-clearing and interveinal chlorotic spotting on the young leaves. On later formed leaves the veins are severely puckered, resulting in dark green blisters. The leaves are small and the plant is stunted.

With the mild strain there is a veinclearing of the young leaves, followed by a chlorotic mottle and slight crinkle on the next formed leaves. After several months, only the base of the new leaves is a lighter green than normal with dark green along the veins. The plant is only slightly stunted. PLATE 3.

Nicotiana rustica. There is no local reaction. Both isolates induce a veinclearing on the young leaves followed by a chlorotic mottle with short dark green veinbands and irregular islands. The leaf surface is rugose with both convex and concave blisters. The leaves are elongated.

Nicotiana sanderae. No local reaction develops. With both strains the first symptoms are a slight puckering of the veins and rolling of the leaf margins. Later a chlorotic mottle with irregular dark green islands develops.

After a month, the severe strain causes a necrosis of the veins, which results in a distortion of the lamina.

Nicotiana sylvestris. There is no local reaction nor preliminary veinclearing or chlorotic spotting. The young leaves are a lighter green with dark green rectangles forming an angular mosaic. They are slightly puckered.

Nicotiana tabacum var. White Burley. There is no local reaction. In 8–10 days a chlorotic network appears on the young leaves, followed by a chlorotic mottle with short dark green veinbands. On later formed leaves there are large chlorotic blotches and irregular dark green islands. The laminae are slightly crinkled and the margins are waved.

With the severe strain the chlorotic and dark green symptoms are accentuated, and there is a distortion of one half of the lamina causing the leaf to curve towards that side. These leaves are also elongated and the plant is stunted.

Petunia hybrida. No local reaction develops. The first systemic symptom with the severe strain is a clearing of the veins of the young leaves in 7 days, followed by a chlorotic speck mottle in 2 weeks and then speckled ring patterns. These patterns become almost white as the leaves mature. On new secondary shoots there is a shock reaction of necrotic areas which soon turn white. These areas may fuse and the leaves become flaccid. Later formed leaves on these shoots are elongated and malformed. There is a distinct colour break on most flowers.

With the mild strain there are only a few chlorotic blotches and later chlorotic speck patterns.

Physalis peruviana. There is no local reaction. After 18–20 days areas of chlorotic network appear on the young leaves, but these soon fade. After several months there is an indistinct dark green mottling of some leaves.

GROUP B

SOLANACEAE

Capsicum spp. There is no local reaction. After several weeks the young leaves show isolated chlorotic flecks and dark green islands. The flecks enlarge and become vivid bright yellow. If they are adjacent to the midrib, this bends down at right angles at that point.

Lycopersicon esculentum. No local reaction. Isolated irregular vivid yellow areas develop after 6–8 weeks, usually along the veins. Most leaflets are severely curled back and there may be a diffuse mottle.

DISCUSSION

A survey of the literature shows that several virus diseases of *Datura* spp. have been reported—some found primarily on *Daturas*, others more often on other host plants in the *Solanaceae*.

1. *Datura virus* 1 (Smith & d'Oliviera in Smith 1937) does not cause any malformation on *Datura* spp. and is able to infect *Phaseolus* and *Vigna* whereas this virus is apparently confined to the solanaceous plants.

2. *Datura virus* 2 (Thomas & Krishnaswami 1939) is not sap transmissible whereas this virus does not even need the addition of an abrasive.

3. *Datura virus* 3 (*Datura* distortion mosaic virus) (Capoor & Varma 1948, 1952) does not infect *D. stramonium* systemically, although the symptoms on *D. innoxia* do include malformation. Capoor's virus caused a systemic necrosis of White Burley but most other *Nicotianas* showed only a local necrotic reaction.

4. *Datura virus* 3a (Garga 1958) is confined to *Datura* spp. and petunia while this virus can infect several other host plants.

5. *Datura rugose leaf curl virus* (Grylls 1954) is transmitted solely by leafhoppers.

6. *Datura necrosis virus* (Badami & Kassanis 1959) as its name implies induces a lethal local and systemic necrosis on *D. tatula* and severe mosaic and stunt on tobacco.

7. *Pepper veinbanding virus* }
 8. *Eggplant mosaic virus* } (Dale 1954, 1956). Both infect a large number of solanaceous plants but *Datura* spp. were not included in the host range. Nevertheless, symptoms on other plants, hosts and insect vectors differ from those described for this virus.

9. *Solanum phureja virus* (Bagnall 1963) does not infect *Datura* spp., tobacco, *N. glutinosa* or *Capsicum*.

10. *Datura Quercina virus* }
 11. *Datura Z-virus* } (Blakeslee 1921). Both caused severe malformation of *Datura* leaves and the suppression of the spines on the capsules. With the methods used at that time Blakeslee was unable to sap-transmit the Quercina virus, although he did graft-transmit it to other *Daturas*, but not to tobacco, tomato, petunia, etc. Furthermore, this Q-virus was seed transmitted to 79 per cent of the progeny of *Datura* spp., whereas the virus described in this paper is definitely not seed-borne.

The Z-virus was easily sap-inoculable and not seed transmitted; but tobacco was not a host. Nevertheless, Smith (1957) places this Z-virus in the tobacco etch virus group.

12. *Tobacco etch virus group* (Johnson 1930). A comparison of this *Datura* virus with those in the tobacco etch virus group shows some similarities with several, but no complete correlation with any one of the present strains.

Bawden (1964) cites the suppression of spines on the seed pod as a feature of tobacco etch virus on *Datura*. However, the necrotic etch symptom on tobacco which is a characteristic of the viruses in this group does not occur with the virus described here. The V-strain of Stover (1951) is the only one which, under greenhouse conditions, does not induce systemic necrotic etch on tobacco, only a mild mottle and veinbanding; but on *Daturas* the V-strain causes only a transient chlorotic spotting and no malformation. Even the mild strain of Bawden & Kassanis (1941) causes an initial etching on tobacco which later fades.

Doolittle & Alexander (1951) working on tobacco etch in greenhouse tomatoes which showed a mild mottle and curl similar to that caused by this *Datura* virus, state only that all isolates induce "severe malformation" of *D. stramonium* leaves.

The pepper strain of Greenleaf (1953) resulted in a very severe wilt of *Capsicum* spp. and is thereby excluded from this comparison. However, the bell pepper strain of McLean (1962) does cause distortion of *D. stramonium* leaves but the typical necrotic etch occurs on tobacco.

A comparison with the host range of tobacco etch virus as given by Holmes (1946) shows many similarities in the susceptible species, but not in the symptoms induced on these species.

Hollings (1959) describes the symptoms of tobacco severe etch virus on a new indicator test plant, *Nicotiana clevelandii*, as a yellow network followed by a chlorotic mottle, dwarfing and rosetting. In contrast, this *Datura* virus causes such severe malformation that the leaves are reduced to strings with occasional dark green blisters.

CONCLUSIONS

The *Datura* virus described above appears to be a new virus or strain belonging to the tobacco etch virus group, even though necrotic etching does not occur on tobacco. Suppression of the spines on *Daturas* is a feature of both this virus and tobacco etch. According to Rossouw (private communication) tobacco etch has never been isolated from tobacco in South Africa, and the occurrence of a virus in this group is thus a new record for this country.

The malformation of *Datura* leaves is extremely severe with this new virus, and a similar malformation occurs on *N. clevelandii* leaves. Therefore, the name DATURA MALFORMATION VIRUS is suggested.

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PLATE 1.—Left: Healthy *Datura* plant. Centre and right: Severe strain showing malformation of leaves, distortion of flower and spineless pod. Sap transmission.



PLATE 2.—Left: Severe strain on *Datura* showing string-like leaves. Aphid transmission. Right: Mild strain showing distorted chlorotic areas. Sap transmission.



PLATE 3.—Top: *Nicotiana langsdorffii*. Sap transmission. Left: Mild strain. Right: Severe strain.
Bottom: *Daturas*, showing almost complete suppression of spines on the pods.



PLATE 4.—*Nicotiana clevelandii*. Sap transmission. Left: Severe strain. Right: Mild strain.
Healthy plant in the centre.