# A NEW VIRUS DISEASE OF CARROTS: ITS TRANSMISSION, HOST RANGE, AND CONTROL

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(Plates 1-4)

[*Manuscript received May* 26, 1948]

*Summary*

A new virus disease of carrots, which was recorded in Melbourne in 1943, is described. The disease occurs throughout Victoria, and has also been recorded in New South Wales, South Australia, Western Australia, and Tasmania.

The disease caused the virtual abandonment of early carrot production in the Melbourne vegetable area and, during the last war, was the principal limiting factor to carrot seed production.

In Victoria, carrot crops are exposed to infection during the period April to December, but a disease-free period occurs during the summer months.

Infected crops have a stunted, unthrifty appearance suggestive of mineral deficiency. Foliage symptoms consist of an irregular chlorotic mottle and marginal reddening of the lower leaves. Leaflets are distorted and reduced in size, and petioles and subpetioles are twisted longitudinally. Growth stunting is severe in susceptible varieties. Stem necroses sometimes develop on the youngest leaves of fully grown, recently infected plants, and on the older leaves when infection is of long duration.

The virus is transmitted by the aphid *Cavariella aegopodii,* which is a serious pest of carrots; but not by the aphids *Hyadaphis foeniculi, Anuraphis tulipae, Myzus persicae,* nor *Macrosiphum* spp. The Jassids, *Thamnotettix argentata, Erythoneura ix,* and *Empoasca* sp., also failed to transmit the virus.

The virus has been transmitted by core-grafting, but not by mechanical inoculation methods. There is no evidence of transmission through carrot seed.

Carrot is the only known natural host plant of the virus, but *Apium ammi, A. australe,* hemlock, dill, and coriander have been experimentally infected. Celery, parsley, parsnip, caraway, and fennel appear to be immune to the virus.

A wide range of cultivated varieties and wild strains of *Daucus carota* developed infection when grown under field conditions, but several local varieties were tolerant to the disease.

The vector, which is widely distributed in Victoria, occurs commonly on willow and fennel, and is a serious pest of carrot, celery, parsley, and parsnip.

Histological studies of the feeding habits of the vector showed that it was a phloem feeder.

The virus is of a persistent type and, in one experiment, the vector, after an infection feeding period of forty-eight hours remained infective during eighteen days of serial transfers. There appears to be no evidence of the occurrence of a latent period.

In two controlled experiments virus infected roots were subject to high mortality when transplanted for seed, whereas all healthy roots produced vigorous seed plants. Surviving infected seed plants lacked vigour and seed production was greatly reduced.

The invert sugar content of healthy roots was significantly higher than that of infected roots.

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Virus infected and healthy roots rotted at the same rate when wound inoculated with fungal organisms which were associated with root decay in Victorian seed crops.

It is believed that the biological breakdown of transplanted infected roots is frequently preceded by a form of necrotic collapse directly attributable to the virus.

Environmental control of the vector, and the disease, has been obtained by delaying the sowing of susceptible varieties until early summer.

In a spraying trial with spring sown Chantenay carrots, weekly applications of a proprietary DDT emulsion almost completely eliminated the vector, significantly reduced the amount of disease, and resulted in a sixfold increase in yield of marketable roots.

Disease control has also been demonstrated by comparing the infected yields of several virus tolerant varieties, and one susceptible variety, grown under experimental conditions. A Victorian virus tolerant selection out-yielded other varieties in this trial.

## I. Introduction

During 1940 commercial vegetable growers in the Melbourne market-garden area requested an investigation of a disease of spring sown carrots, which had been occurring over a number of years. Summer sowings were not affected, but the failure, or partial failure, of spring sowings had made the production of this crop uneconomic, and as a result, the continuity of carrot supplies to the Melbourne market had been disrupted.

The principal objects of the investigation described in this paper have been the elucidation of the factors responsible for the disease, and the development of practical disease control measures.

A preliminary account of the investigation, which commenced in 1941, has been reported elsewhere (Stubbs and Grieve 1944).

## II. History of the Disease

According to some experienced vegetable growers, spring carrot production was abandoned in many of the older suburban market-gardens about thirty years ago. The soil was said to have become "carrot sick" in these areas.

However, the disease was not reported until the spring of 1938 when crop failures occurred in the newer market-gardens on the outskirts of the city area. These sparsely settled districts probably owed their earlier freedom from the disease to their isolation, and it is a logical assumption that the disease followed the population trend from the inner to the outer suburbs as home gardens supply continuous reservoirs of infection.

During the last war, when carrot root and seed production was greatly increased, the disease appeared in isolated rural districts in which carrots were not formerly grown. It is thought that the disease was introduced to these districts by infected seed-roots brought from established vegetable areas.

In the early years of war-time seed production a high incidence of root-rot resulted in the failure of most carrot seed crops grown in Victoria. These failures were eventually attributed to the transplanting of infected roots, and were largely overcome by the selection of healthy root crops for seed production.

## III. Identification of the Disease

In surveys of metropolitan market-gardens during the spring and summer of 1941-42 all spring sown carrot crops were found to be affected. These crops were uniformly stunted and had an unthrifty chlorotic appearance suggestive of a nutritional disorder.

The symptoms of the disease resembled those described by Warington (1940) for carrots grown in water culture medium deficient in boron. Her experiments were duplicated by the author, and the symptoms shown by boron deficient carrots were found to be very similar to those occurring in field crops.

At this stage of the investigation a lime-induced boron deficiency appeared to be a possible cause of the disease, and during 1941 and 1942 nutritional experiments were carried out with boron and other trace and major elements, but with negative results.

In the course of these experiments it was noticed that the appearance of disease symptoms was preceded or accompanied by infestations of various species of leaf-hoppers and aphids which, in view of the failure of the nutritional experiments, suggested the possibility of the disease being caused by an insect transmitted virus.

At that time virus diseases of carrots had only been recorded in the United States, where both the Aster Yellows virus (Zundel 1929; Severin 1930) and the Western Celery Mosaic virus (Severin and Freitag 1938) caused economically important diseases. Neither of these viruses had been recorded in Australia.

In preliminary virus transmission experiments, healthy carrot seedlings and various indicator plants (*Nicotiana tabacum, Datura stramonium,* etc.) were mechanically inoculated with sap expressed from diseased plants, but negative results were obtained.

In November 1942, insects occurring in diseased carrot crops were collected and identified; of these the Jassid *Thamnotettix argentata* Evans was a known vector of a virus disease (Hill 1941). During the summer of 1942-43 adult specimens of *T. argentata* were caged on the foliage of diseased carrots where they bred readily. The adult progeny of these Jassids were fed on the foliage of healthy carrots, but the plants remained healthy. Negative results were also obtained when similar transfers were made with the leaf-hoppers *Erythroneura ix* Myers and *Empoasca* sp.

Commencing on September 1, 1943, fortnightly sowings of carrots were made in an insect-proof field cage and concurrently in an area adjacent to this cage, at Burnley. The plants grown outside the cage became heavily infested with one species of aphid, and developed typical disease symptoms in November, whereas those grown within the cage remained vigorous and continued to produce normal foliage.

On November 20, 1943, groups of aphids from the affected plants were caged on single leaves of each of six semi-mature carrots within the field cage. The aphids were allowed to feed for seven days before being removed together with the leaves on which they had been feeding. Twenty-one days after the introduction of the aphids a mosaic mottle developed on the youngest fully

emerged leaves of these plants. Symptoms identical with those shown by the plants outside the cage soon appeared and, as the surrounding uninoculated plants remained healthy, the suspected virus origin of the disease was confirmed.

Specimens of the aphid used in this experiment were identified by E. H. Zeck, Department of Agriculture, N.S.W., and by the Imperial Institute of Entomology, London, as *Cavariella aegopodii* Scopoli.

## IV. Distribution

The disease has been recorded throughout Victoria and in New South Wales, South Australia, Western Australia, and Tasmania. The known distribution of the disease in Victoria is shown in Figure 1.


Fig. 1.—Known distribution of the carrot virus disease in Victoria.

## V. Seasonal Occurrence

In all districts the disease cycle approximates very closely to the aphid cycle. The vector is favoured by the moist, cool conditions of spring and autumn, but it is unable to survive even the moderate summer temperatures of a normal season in southern Victoria. The aphid appears to be less sensitive to low temperatures, and light populations may persist on carrots, or other hosts, throughout the winter. However, a combination of low temperatures and heavy continuous rains is not conducive to its development. In the spring the vector is extremely active on many Umbelliferous species, and it is during this period, probably owing to the prevalence of winged forms, that virus spread is most rapid. In a plot of spring sown carrots it is almost impossible to record the rate of spread of the virus. The initial appearance of winged aphids is followed by the development of a few infection foci within a plot, and in a week or ten days symptoms appear almost simultaneously on the remainder of the plants. In the

autumn the aphids are more sluggish, winged forms are less prevalent, and the rate of virus spread is considerably reduced.

The duration of the infestation is dependent upon the climatic environment of the locality and upon current seasonal conditions. During two seasons (1943-44 and 1944-45) disease surveys showed that all carrot crops sown at Orbost and Bruthen prior to the first week in December became infected. In the drier Lindenow district November sowings were free from virus, but October sowings were heavily infected. In the metropolitan area carrots sown after the second week in December remained healthy. In north-western Victoria (Mildura, Swan Hill), where carrots are grown as a winter crop, an autumn and winter infestation is of more importance than a spring infestation: the latter is of relatively short duration because of the higher temperatures experienced in this area.

## VI. Symptoms

In spring or early summer an infected carrot crop has a uniformly unthrifty appearance which is more typical of a physiological disorder than of a virus disease.

The foliage of infected plants is dwarfed relative to that of healthy plants and the older leaves exhibit an irregular chlorotic mottle which may be accompanied by marginal reddening. These latter symptoms, which vary considerably from plant to plant, and according to the age of the plant at the time of infection, resemble the autumn tinting of deciduous plants (see Plate 1, Fig. 1).

The individual leaflets of infected plants are distorted, and reduced in size, and their surfaces are inclined in various planes due to longitudinal twisting of the petioles and subpetioles (see Plate 1, Fig. 2). The petioles of the older leaves are sometimes bent in an "S"-shaped manner (see Plate 1, Fig. 3).

With the advent of high temperature conditions, symptom expression gradually decreases in intensity, and foliar mottling may be completely masked. The writer, while searching for healthy crops for seed production purposes, experienced difficulty in detecting infected crops during the summer period. Symptom masking was most complete in dry-farming areas subject to hot dry conditions in midsummer.

When, however, a healthy crop grown under these conditions is viewed alongside an infected crop with masked symptoms, distinct differences in size and colour of foliage become apparent. Healthy carrot foliage of the Chantenay variety approximates closely in colour to a dark, dull yellow green and infected unmottled foliage to grass green or cress green (Ridgway 1912). The individual leaflets of infected plants are also considerably smaller than those of healthy plants. Unfortunately, these differences are of a relative nature and are, therefore, not reliable diagnostic aids.

Further evidence in support of the suggested relationship between air temperature and symptom masking is provided by the behaviour of infected plants grown under glass-house conditions. In winter, when maximum temperatures rarely exceed 70°F. and diurnal variations are of the order of 20-25°F., experimentally infected carrots develop intense foliage mottling, and are very dwarfed relative to healthy plants (see Plate 2, Fig. 1). In spring, summer, and

autumn, when maximum day temperatures frequently exceed 85°F., and the diurnal range is smaller, leaf mottling is difficult to detect, and dwarfing is less severe.

Mature summer-sown carrots, which usually become infected in autumn or early winter, develop a severe and characteristic reaction to the virus. The petioles of leaves emerging subsequent to infection are twisted, brittle, and reduced in length, and sometimes exhibit brown necrotic streaks. Leaflets are distorted, reflexed, and distinctly mottled, and the youngest leaves frequently become rosetted (see Plate 1, Fig. 4).

In spring-infected crops, which remain unlifted until the autumn, foliage mottling and distortion are more severe than during the early infection period. Stem necroses, as above, appear on the lower leaves which progressively wither and die. This latter symptom appears to be associated with mature plants, and has never been observed on young plants of any carrot variety. When mature plants, which had been infected for a long period, were moved to a heated glasshouse during the winter, stem necroses developed rapidly on the lower leaves, and the plants died within a few weeks.

## VII. Materials and Methods

Colonies of non-infective *C. aegopodii* were maintained either on carrot or fennel (*Foeniculum vulgare* Gaertn.). The latter plant is immune to the virus, very palatable to the vector, and capable of supporting heavy aphid populations for longer periods than carrot. A semi-permanent colony was maintained on fennel plants grown in a field cage, which was shaded from all but the morning sun. This colony survived two summers.

The aphids bred on fennel were fed on carrot for a week or more before being transferred to infected plants. This precautionary procedure was adopted to overcome any possibility of reduced infectivity of the vector which might result from feeding on the sap of an immune plant.

Short term colonies of aphids were maintained satisfactorily beneath cylindrical, muslin or cellophane covered cages, constructed according to the details supplied by Hamilton (1930). Moisture-permeable cellophane was found to be the most suitable covering material except during the summer when it contracted and split very readily.

Field cages used for the propagation of test plants were originally covered with cotton marquisette, treated with a rot-proofing solution containing copper oleate or copper naphthenate. This material was more resistant to weathering than other cotton fabrics tested, but usually required replacement after two years' exposure. A more permanent type of field cage is covered with brass wire gauze (40 mesh, 34 gauge) and Windowlite (Hill 1941), a proprietary glass substitute consisting of galvanized fly-wire impregnated with a cellulose compound. The gabled roof, and top three feet of the walls of this cage, are covered with Windowlite, and the lower section of the walls with wire gauze. The cage is provided with an insect-trap entrance.

Test plants of most Umbelliferous species were grown satisfactorily in six-inch flower pots in which drainage was provided by a layer of wood charcoal.

The plants were raised in steam sterilized soil and the same soil type was used in all transmission experiments. The normal procedure was to sow seed in four equidistant clumps and thin each clump to a single seedling prior to inoculation.

In insect transmission experiments, aphids were caged on seedling plants by means of celluloid cylinders covered at one end with marquisette. These feeding cages were constructed from celluloid sheets rendered malleable by dipping in hot water, and welded together with amyl acetate or acetone. The marquisette tops were also welded in place with either of these solvents.

Aphids were transferred from plant to plant by means of a fine camel-hair brush. When air temperatures were low, it was difficult to induce adult forms of *C. aegopodii* to stop feeding, thus making transfers time-consuming and tedious. The aphids were rapidly activated by subjecting infested foliage to a temperature of 80-85°F. for an hour or more. Aphids incubated at this temperature overnight did not show any apparent loss of infectivity.

In order to avoid migration of aphids during the transfer process three plants in each pot were always covered with the feeding cages. Alternate plants were inoculated with infective and non-infective aphids respectively. The number of aphids introduced, and the length of the feeding periods varied according to the nature of the experiment. In host range studies twenty aphids were transferred to each plant and allowed to feed for at least forty-eight hours. The plants were then fumigated and removed to an insect free cage.

Injury to plant tissues sometimes occurred when heat-volatilized nicotine was used as a fumigating agent, and a method was devised whereby plants enclosed in the feeding cages were dusted with a commercial three per cent. nicotine dust without the necessity of removing the cages (Stubbs 1946).

## VIII. Transmission

### (*a*) *Insect Transmission*

The efficiency of *C. aegopodii* in transmitting the virus to carrots and other susceptible Umbelliferous hosts has been demonstrated in numerous experiments. In all experiments where a minimum of ten and a maximum of twenty viruliferous aphids have been fed on virus susceptible test plants, for periods ranging from twenty-four to forty-eight hours, 100 per cent. infection has occurred. The incubation period of the virus in carrot averaged seventeen days, and the shortest time recorded for the development of symptoms was fourteen days.

Table 1

EFFICIENCY OF SINGLE APHIDS IN TRANSMITTING THE CARROT VIRUS

| | Stage of Development of *C. aegopodii* | Plants Infected (10 inoculated) (%) |
|---|---|---|
| 1 | Alate females | 90 |
| 2 | Pre-alates with wing-buds | 40 |
| 3 | Second instar pre-alates | 30 |
| 4 | Viviparous apterous females | 60 |

In one experiment, separate groups of twenty infective *C. aegopodii* were fed on carrot seedlings for 10 min., 30 min., 1 hr., 2 hr., 3 hr., and 24 hr. The plants were dusted at the end of each feeding period. All plants became infected with the exception of those exposed to aphids for the 10-min. period.

In another experiment, carrot seedlings were exposed to single infective aphids in the various stages of development listed in Table 1.

### (*b*) *Insects which Failed to Transmit the Virus*

Failure to transmit the carrot virus with the Jassids *Thamnotettix argentata* Evans, *Erythroneura ix* Myers, and *Empoasca* sp., was reported earlier in this paper.

Aphids other than *C. aegopodii* which commonly infest, and are capable of breeding on, carrots include *Anuraphis tulipae* Boyer, *Hyadaphis foeniculi* Pass., *Myzus persicae* Sulzer, and *Macrosiphum* spp.

None of these species was able to transmit the virus when groups of twenty were transferred from infected to healthy carrots. The technique used in these experiments (Table 2) was identical with that used in transmission experiments with *C. aegopodii,* and in most cases control transfers were carried out with this aphid.

TABLE 2

VIRUS TRANSMISSION EXPERIMENTS WITH APHID SPECIES WHICH BREED ON CARROTS

| Exp. No. | Aphid Species | Source | Results* |
|---|---|---|---|
| 1 | *Hyadaphis foeniculi* | Mixed colonies on infected carrot | 0/8 |
| | *C. aegopodii* | | 8/8 |
| 2 | *Anuraphis tulipae* | Mixed colonies on infected carrot | 0/4 |
| | *Hyadaphis foeniculi* | | 0/4 |
| | *C. aegopodii* | | 4/4 |
| 3 | *Macrosiphum* sp. 1 | Mixed colonies on infected carrot | 0/4 |
| | *Macrosiphum* sp. 2 | | 0/4 |
| | *C. aegopodii* | | 4/4 |
| 4 | *Anuraphis tulipae* | Infected carrot | 0/8 |
| | | Healthy carrot | 0/8 |
| 5 | *Myzus persicae* | Infected carrot | 0/10 |
| | | Healthy carrot | 0/10 |

* The numerator refers to the number of plants infected and the denominator to the number inoculated.

### (*c*) *Graft Transmission*

In this experiment the foliage of a number of semi-mature healthy carrots was removed by transverse cuts at the bases of the petioles. Holes were then bored in each root by means of a sterile cork borer inserted at right angles to the long axis and passed completely through the central tissues to the opposite side of the root. Cores obtained in a similar fashion from infected roots were then inserted aseptically into the healthy roots, using the method described by

Murphy and M'Kay (1926). The surfaces of the grafts were sealed with melted microcrystalline paraffin wax. Controls consisted of healthy ungrafted roots and roots grafted with plugs from healthy carrots.

After completion of the grafts the roots were partly buried in trays of moist sand and stored in the saturated atmosphere of a glass-house humidity chamber for a period of four days. The average temperature for this period was 72°F., with a minimum of 60°F. and a maximum of 90°F. The roots were then planted in pots of sterile soil. Each pot contained a root grafted with an infected plug and either a healthy grafted or ungrafted root. The pots were retained in the humidity chamber for a further period of twenty-seven days and were then transferred to a field cage, as glass-house temperatures were too high for optimum symptom development. Eight days after their removal to the field cage, virus symptoms were observed on roots grafted with infected plugs. The plants were kept under observation for several weeks and, during this period, the foliage of the infected carrots became very dwarfed and intensely mottled.

At the conclusion of the experiment the roots of all the grafted plants were sectioned longitudinally and the graft unions examined. A high percentage of the grafts had knitted completely with the surrounding tissues (see Plate 3, Fig. 1), but graft unions had not occurred in those plants which failed to develop symptoms.

The results of the experiment were as follows:

| | |
|---|---|
| Infected core grafted to healthy root . . . | 14 infected of 18 grafted |
| Healthy core grafted to healthy root . . . | 9 healthy of 9 grafted |
| Ungrafted healthy roots (18) . . . . . | 18 healthy |

*(d) Failure to Transmit the Virus by Mechanical Methods of Inoculation*

Negative results were obtained in all experiments where the leaves of healthy carrot seedlings, and several non-Umbelliferous virus indicator plants, were rubbed, with and without abrasive (aloxite, 600 mesh), with undiluted sap extracted from the leaves or roots of infected carrot plants. An extract from large numbers of infective aphids also failed to infect carrot.

The addition of a reducing agent, sodium sulphite (Bald and Samuel 1934), to the inoculum, did not induce symptom development in carrot, nor did infection occur when inocula, prepared from leaves of infected carrot and slender celery extracted with dipotassium phosphate and 0.1M phosphate buffers at various pH levels, were used (Thornberry 1935; Stanley 1936). The details of these experiments are recorded in Table 3. In Experiment 5 (Table 3) inocula were prepared by grinding 2 g. samples of leaf tissue with 10 ml. aliquots of buffer solution.

*(e) Failure to Transmit the Virus through Carrot Seed*

On three separate occasions, a number of samples of seed gathered from infected and healthy carrots was planted in short rows in a field cage. There were no apparent differences in vigour between plants grown from the various samples, and virus symptoms did not appear.

TABLE 3

SAP INOCULATION EXPERIMENTS

| Exp. No. | Source and Type of Inoculum | Method of Inoculation | Variety Inoculated | Results* | |
|---|---|---|---|---|---|
| 1 | Undiluted sap from leaves of naturally infected carrots | Leaf rubbing without abrasive | Danvers | 0/6 | |
| | | | Nantes | 0/6 | |
| | | | Morse's Bunching | 0/7 | |
| | | | Imperator | 0/11 | |
| | | | Oxheart | 0/8 | |
| | | | Chantenay | 0/8 | |
| | | | | Inoculum A | Inoculum B |
| 2 | Sap from leaves of naturally infected carrots. Inoculum A diluted 1/1 with 0.1% solution of anhydrous $Na_2SO_3$. Inoculum B undiluted sap | Leaf rubbing with abrasive | Chantenay | 0/50 | 0/50 |
| | | | Danvers | 0/5 | 0/5 |
| | | | Nantes | 0/7 | 0/7 |
| | | | Morse's Bunching | 0/6 | 0/6 |
| | | | Imperator | 0/8 | 0/8 |
| | | | Oxheart | 0/6 | 0/6 |
| 3 | Sap from leaves of naturally infected immature carrots | Leaf rubbing with abrasive | Chantenay | 0/70 | |
| | | | Oxheart | 0/5 | |
| | | | Nantes | 0/5 | |
| | | | Imperator | 0/6 | |
| | | | Danvers | 0/5 | |
| | | | Morse's Bunching | 0/5 | |
| | | | *Datura stramonium* | 0/6 | |
| | | | *Nicotiana tabacum* | 0/6 | |
| | | | *Solanum nigrum* | 0/6 | |
| | | | Tomato | 0/4 | |
| | | | *Vigna sinensis* | 0/6 | |
| | | | *Phaseolus vulgaris* | 0/6 | |
| 4(*a*) | Sap from roots of infected carrots | Leaf rubbing with abrasive | Chantenay | 0/8 | |
| 4(*b*) | Water extract from infective *C. aegopodii* | Leaf rubbing with abrasive | Chantenay | 0/10 | |
| 5(*a*) | Sap from leaves of infected carrot extracted with 3% by wt. $K_2HPO_4$. Reaction adjusted to pH 7.1 with 0.1M phosphate buffer at pH 7.0 | Leaf rubbing with abrasive | Chantenay | 0/20 | |
| 5(*b*) | Sap from leaves of infected carrot extracted with 0.1M phosphate buffer at pH 7.0. Reaction of buffered extract pH 6.7 | Leaf rubbing with abrasive | Chantenay | 0/20 | |

TABLE 3 (continued)
SAP INOCULATION EXPERIMENTS

| Exp. No. | Source and Type of Inoculum | Method of Inoculation | Variety Inoculated | Results* |
|---|---|---|---|---|
| 5(*c*) | Sap from leaves of infected carrot extracted with 0.1M phosphate buffer at pH 8.0. Reaction of buffered extract pH 7.3 | Leaf rubbing with abrasive | Chantenay | 0/20 |
| 5(*d*) | Sap from leaves of infected carrot extracted with distilled water. Reaction of extract pH 6.3 | Leaf rubbing with abrasive | Chantenay | 0/20 |
| 5(*e*) | Sap from leaves of healthy carrot extracted with distilled water. Reaction of extract pH 6.4 | Leaf rubbing with abrasive | Chantenay | 0/20 |
| 5(*f*) | Sap from leaves of infected slender celery extracted with 3% by wt. $K_2HPO_4$. Reaction adjusted to pH 7.2 with O.1M phosphate buffer at pH 7.0 | Leaf rubbing with abrasive | Chantenay | 0/20 |
| 5(*g*) | Sap from leaves of infected slender celery extracted with 0.1M phosphate buffer at pH 8. Reaction of buffered extract pH 7.5 | Leaf rubbing with abrasive | Chantenay | 0/20 |
| 5(*h*) | Sap from leaves of healthy slender celery extracted with distilled water. Reaction of extract pH 6.7 | Leaf rubbing with abrasive | Chantenay | 0/20 |

* The numerator refers to the number of plants infected and the denominator to the number inoculated.

The writer has been unable to find evidence of seed-transmission in field crops, in spite of the fact that most of the carrot seed used in Victoria during the later stages of the war was produced from virus infected seed crops. Many of the root crops inspected were grown during the period when the vector was absent, and under conditions where virus infected plants would have been detected very readily.

## IX. HOST RANGE

### (*a*) *Natural Host Plants*

The cultivated carrot appears to be the only economically important Umbelliferous host of the virus in Victoria, as disease symptoms have never been

observed on celery, parsley, or parsnip grown in close proximity to infected carrots.

A wild strain of *D. carota,* which is widely distributed in southern Victoria, is the only naturally infected weed host yet discovered.

### (*b*) *Experimentally Infected Host Plants*

The virus was successfully transferred, by means of the vector, to all available varieties of cultivated and wild carrots, and to the following additional Umbelliferous species: Slender celery (*Apium ammi* (Jacq.) Urb.); sea celery (*Apium australe* Thon.); hemlock (*Conium maculatum* L.); dill (*Anethum graveolens* L.); and coriander (*Coriandrum sativum* L.).

The following species did not develop symptoms when exposed to infective aphids: Parsnip (*Pastinaca sativa* L.); celery (*Apium graveolens* L.) var. Golden Self Blanching, White Plume; caraway (*Carum carvi* L.); fennel (*Foeniculum vulgare* Gaertn.); and parsley (*Petroselinum hortense* Hoffm.) var. Triple Curled.

It seems unlikely that any of the above plants are symptomless carriers of the virus as attempts to recover the virus from them have always been unsuccessful. However, it was demonstrated that viruliferous aphids remained infective for several days when fed on celery, parsley, and fennel before being transferred to carrot plants.

*C. aegopodii* fed readily on all the species tested with the exception of hemlock, which appeared to be unpalatable. Infective aphids usually fed on hemlock for a sufficient time to transmit the virus to that plant, but the virus was recovered with difficulty owing to the fact that the aphids usually left the plants after a short initial feeding period, and mortality was high when they were caged on hemlock for more than one or two days. The virus has not yet been recovered from sea celery, possibly because the virus concentration in this plant is very low.

## X. Symptoms on Experimentally Infected Host Plants

*Slender celery.*—This weed, which is native to Australia, is very susceptible to the virus. The earliest symptoms appear on the youngest leaves and consist of chlorosis and recurving of the leaflets accompanied by epinasty of the petioles. Symptoms have been observed eleven days after exposure to viruliferous aphids (see Plate 2, Fig. 2).

Following the development of primary symptoms severe stunting of growth occurs and infected plants frequently die as a result of a progressive necrosis which usually commences in the lower stem region.

Slender celery is the best differential host of the virus yet discovered, as symptoms are readily discernible and are not subject to masking.

*Sea celery.*—When seedlings in the second true leaf stage were exposed to viruliferous aphids a mild mottling developed in the lower leaves approximately fifty days after inoculation. These symptoms did not increase in intensity and neither foliage distortion nor growth stunting occurred.

*Coriander.*—Chlorosis and reddening of the foliage was accompanied by severe stunting and infected plants usually died. This plant is a recent addition to the host range of the virus, and its reaction has not yet been studied in detail.

*Hemlock.*—Symptom development is very rapid. The earliest symptoms, consisting of chlorotic and necrotic flecking of the lower leaves, have been observed eleven days after exposure to the vector. These symptoms become more pronounced about thirty days after inoculation. The leaflets become slightly reflexed and irregular chlorotic patches appear on the petioles, which become twisted or bent in a similar fashion to that observed in carrot. The growth rate of infected plants is slightly retarded.

*Dill.*—Infected plants were severely dwarfed and developed a purplish-red coloration of the lower leaves.

*Wild carrot.*—Wild forms of *D. carota* collected in the Dandenong Ranges, in south and east Gippsland, and in south-eastern New South Wales, were all susceptible to the virus. When grown under similar conditions to the cultivated carrot, these forms produced a rosetted type of foliage growth and white forked roots.

Foliage symptoms on seedling plants were less obvious than those on cultivated varieties, but growth stunting was equally severe. Leaflet distortion, petiole twisting, and mosaic mottling were not obvious, but a characteristic red pigmentation occurred in the petioles and margins of the lower leaves. This latter symptom was more pronounced when larger plants were infected.

## XI. Reactions of Cultivated and Wild Varieties of Daucus carota to the Virus

A number of named carrot varieties, and several unnamed selections and wild strains of *D. carota,* were exposed to natural infection under field conditions. Field trials were usually sown in spring, but supplementary sowings were sometimes made in autumn. These trials were conducted over a three-year period, but not all the varieties listed in Table 4 were available for testing during this period.

The reactions of individual varieties to the virus were assessed by the degree of foliage distortion, mottling and dwarfing, and root size at the time of lifting. In these trials lack of foliage vigour was always accompanied by a reduction in root size.

All varieties tested were susceptible to infection and, as the majority of the varieties reacted in a similar fashion to the virus, it was not possible to apply any form of disease rating.

Several Australian varieties and selections exhibited marked tolerance to the virus and of these a Victorian selection showed most promise. The history of these varieties is obscure, but it is thought that they originated from selections, made by growers, from vigorous types which sometimes occur in diseased crops of susceptible varieties. The author has observed virus tolerant "strangers" in infected crops of the Chantenay variety, which were characterized by vigorous foliage and coarse Intermediate-type roots.

TABLE 4

REACTIONS OF CULTIVATED AND WILD VARIETIES OF D. CAROTA TO THE VIRUS

| Variety | Origin of Seed | Reaction to Carrot Virus |
|---|---|---|
| Chantenay (4 lines) | U.S.A. | Susceptible |
| Chantenay | England | Susceptible |
| Chantenay | Tasmania | Susceptible |
| Champion Intermediate | W. Australia | Tolerant |
| Danvers | U.S.A. | Susceptible |
| Danvers | England | Susceptible |
| Early Market (2 lines) | England | Susceptible |
| Hutchinsons' | U.S.A. | Susceptible |
| Imperator (2 lines) | U.S.A. | Susceptible |
| Imperator X Champion Intermediate | W. Australia | Tolerant |
| James' Intermediate (Scarlet) | England | Susceptible |
| Johnson's Maincrop | England | Susceptible |
| Long Orange | U.S.A. | Susceptible |
| Morse's Bunching | U.S.A. | Susceptible |
| Nantes | U.S.A. | Susceptible |
| Nantes | Australia | Susceptible |
| Nantes Gribvoskaya | U.S.S.R. | Susceptible |
| Oxheart | U.S.A. | Susceptible |
| Oxheart | England | Susceptible |
| Paris Carotel | U.S.S.R. | Susceptible |
| Red-stalked Intermediate | Australia | Tolerant |
| St. Valery | Australia | Susceptible |
| Stump-rooted Intermediate | England | Susceptible |
| Unnamed selection | Victoria | Tolerant |
| Valeria | U.S.S.R. | Susceptible |
| Scarlet Horn (2 lines) | U.S.A. | Susceptible |
| *D. carota*, wild (2 strains) | India | Susceptible |
| *D. carota*, wild (2 strains) | Baluchistan | Susceptible |
| *D. carota*, wild (3 strains) | Iran | Susceptible |
| *D. carota*, wild (4 strains) | Victoria and N.S.W. | Susceptible |

## XII. THE VECTOR

### (*a*) *Distribution*

*C. aegopodii* is widely distributed in Victoria and is a serious economic pest of carrot, celery, parsnip, and parsley.

The aphid has also been found infesting willows and sea celery growing in situations widely separated from agricultural areas.

### (*b*) *Taxonomy*

The taxonomy of *Cavariella aegopodii* has been described in detail by Theobald (1927), but certain morphological features of the Victorian *Cavariella* resemble more closely those of the aphid referred to by Essig (1938) as *C. capreae* Fab. than those of *C. aegopodii*. In view of this discrepancy specimens taken from carrot and fennel in Victoria were forwarded to Essig, who expressed the opinion that they were identical with the American *C. capreae*. He agreed that there was "reason for some confusion concerning the difference

between *capreae* and *aegopodii*," but suggested adherence to the European in preference to the American terminology.

Theobald, on the other hand, separates *C. capreae* from *C. aegopodii*, but in his description of the former, makes reference to the fact that "Schouteden and some other authors place Scopoli's *Aphis aegopodii* as a synonym" of *C. capreae*.

### (*c*) *Food Plants*

In Victoria, the natural host range of *C. aegopodii* approximates closely to that of *C. capreae* in the United States (Essig 1938), but is considerably wider than that described for *C. aegopodii* in England (Theobald 1927).

Naturally occurring colonies of *C. aegopodii* have been found on the following Umbelliferous species: caraway, carrot, celery, celeriac, fennel, hemlock, sea celery, parsley, parsnip, and slender celery.

The aphid completed its life-cycle on all the above plants with the exception of hemlock. All attempts to breed the vector on this plant were unsuccessful, but naturally infested hemlock plants were observed on one occasion, growing in close proximity to heavily infested carrots. Aphids in all stages of development were found concentrated on the under surfaces of the lower leaves of these plants. The reason for the failure of more recent attempts to establish colonies on hemlock is not known, but this plant is obviously unpalatable to the vector and cannot be regarded as a normal food plant.

The aphid also occurs naturally on willow, principally *Salix vitellina*. When the winter dormancy of this plant was broken by striking stem cuttings under hot-house conditions, colonies of aphids developed on the newly emerged leaves, thus suggesting that *C. aegopodii* overwinters in the egg stage on willow in Victoria. Under field conditions aphids usually appear after bud-burst in the spring and, in some districts, particularly east Gippsland, willow appears to be an important host reservoir of the aphid.

### (*d*) *Feeding Habits of the Vector and Tissues Involved*

The difficulty experienced in inducing the vector to withdraw its stylets when feeding suggested that it was not a surface feeder. This belief was further strengthened by the failure to transmit the virus mechanically, this characteristic being consistent with that of many viruses whose vectors are phloem feeders.

In a histological study of the feeding habit of *C. aegopodii* microtome sections were cut of aphids feeding on carrot foliage (see Plate 3, Fig. 2) using the technique described by Dykstra and Whitaker (1938). Permanent mounts were made of preparations showing stylets which were complete either in single, or in serial sections. The sections were stained with a safranin-light green combination. The most satisfactory results were obtained when sections were stained in a 0.5 per cent. solution of safranin in 47.5 per cent. alcohol for 18-24 hours and counterstained in a 0.2 per cent. solution of light green in 95 per cent. alcohol for 2-3 minutes.

Microscopic examination of the sections revealed that the aphid invariably fed on phloem tissue and that the passage of the stylets was usually intercellular through the cortex (see Plate 3, Fig. 3). The absence of any plasmolysis indicated that feeding did not occur in cortical tissues.

(*e*) *Previous Records of Virus Transmission by* Cavariella aegopodii

The author has been unable to find any record of virus transmission by *C. aegopodii,* but Smith (1937) reported that Severin found *C. capreae* capable of transmitting the Cauliflower Mosaic virus, although the aphid did not breed on cauliflower. Severin and Freitag (1938) also recorded transmission of Western Celery Mosaic virus with this aphid, which bred on celery.

In view of the fact that Essig, as reported above, considers the American *C. capreae* to be identical with the Victorian *C. aegopodii,* there appears to be no justification for claiming a first record of virus transmission for *C. aegopodii.*

## XIII. The Virus in Relation to the Vector

The classification and identification of virus diseases is based largely on symptomatology, host range, virus properties *in vitro,* and virus-vector relationships.

The physical properties of the carrot virus could not be determined because of the failure to effect its mechanical transmission, and it therefore became necessary to study its relationship with the vector.

Insect-transmitted viruses have been classified according to their persistence or non-persistence within their vectors (Bawden 1943), and it has been claimed that some viruses of the former type undergo an "incubation" or "latent period" before their vectors develop infective ability.

It was found that the carrot virus persisted in its vector for long periods, but further experiments were initiated to determine firstly, the length of time the vector remained infective after a known feeding period on an infected plant, and secondly, the existence or otherwise of a latent period.

In all of these experiments non-infective apterous females were transferred, after an initial period of starvation, to single detached leaves of infected Chantenay carrots for a known infection feeding period (IFP). Groups of ten, selected from aphids which were feeding, were then serially transferred at regular intervals to healthy plants, for varying test feeding periods (TFP). Carrot was used initially as the test plant, but was eventually replaced by slender celery, which proved more satisfactory because of its greater susceptibility to the virus.

Early experiments indicated that a latent period occurred, but these results conflicted with those of more recent experiments in which the vector, after infection feeds of 24, 18, and 3 hr., and test feeds of 24, 3, and 1 hr. respectively, transmitted the virus to the first series of healthy transfer plants in each experiment.

The earlier results (which are not recorded) are regarded as being unreliable, and may be attributable to a faulty technique or to a low virus concentration in the plants on which the aphids were fed.

The virus persisted in the vector for long periods when the infection feed was of long duration, and for shorter periods when the infection feeding time was decreased, and higher percentages of test plants became infected as the infection or test feeding times were lengthened. These results, which are recorded

in Table 5, are in agreement with those obtained by Watson (1940), in transmission studies with *Myzus persicae* and the Sugar-Beet Yellows virus.

TABLE 5

PERSISTENCE OF THE CARROT VIRUS IN CAVARIELLA AEGOPODII

| Exp. No. | Test Plant | No. of Replicates | IFP (hr.) | TFP (hr.) | No. of Transfers | No. of Transfers in which Infection Occurred | No. of Days Vector remained Infective |
|---|---|---|---|---|---|---|---|
| 1 | Slender celery | 3 | 48 | 24 | 18 | 18 | 18 |
| 2 | Slender celery | 4 | 24 | 24 | 7 | 7 | 7 |
| 3 | Slender celery | 4 | 18 | 3 | 7 | 7 | 4 |
| 4 | Slender celery | 3 | 3 | 1 | 8 | 7 | 2 |
| | Carrot | 3 | 3 | 1 | 8 | 6 | 2 |

## XIV. Effect of Virus Infection on Carrot Seed Production

### (*a*) *Incidence of Root-Rot in Infected and Healthy Seed Plants*

*Experiment 1.*—A preliminary experiment, designed to elucidate a suspected relationship between virus infection and rotting of carrot roots transplanted for seed, was commenced in June 1944.

Infected and healthy roots of the Chantenay variety were selected from a summer sown crop in a Werribee market-garden. Although the vector was present, only small patches of the crop were infested, and less than 20 per cent. of the plants exhibited foliage mottling. The roots of infected plants were not noticeably smaller than those of apparently healthy plants, indicating that infection was of recent origin. It is possible that some of the supposedly healthy plants selected were incipiently infected as their health status could only be assessed by the presence or absence of foliage mottling. Roots were topped immediately after lifting and dipped in a nicotine solution to destroy any aphids which might be present.

Equal numbers of virus infected and healthy roots of uniform size, free from blemishes and growth cracks, were then planted in single row plots in a recently cultivated pasture area at Burnley. Each plot consisted of thirty-six roots planted equidistantly, and five plots of infected roots were alternated with five healthy plots.

During the winter, foliage growth was very slow and the plants remained free from aphids until September. Unfortunately, at the end of this month the plants became heavily infested with pea mite (*Halotydeus destructor* Tucker), which could not be satisfactorily controlled by frequent applications of nicotine dust or sprays. The experiment was discontinued on October 10, 1944, when it became apparent that the less vigorous foliage of the virus infected plants was being affected more severely by the pea mites than the foliage of the healthy plants.

The results recorded in Table 6 are the summations of weekly counts of completely rotted roots; counting commenced on September 4 and concluded on October 10. These results, although incomplete, indicated that virus infection was a predisposing factor for the rotting of seed-roots.

TABLE 6

INCIDENCE OF ROOT DECAY IN HEALTHY AND VIRUS INFECTED SEED PLANTS

| Plot No. | No. Roots Rotted of 36 Planted | | Rotted | |
|---|---|---|---|---|
| | Healthy | Infected | Healthy (%) | Infected (%) |
| 1 | 1 | 7 | 2.8 | 19.4 |
| 2 | 1 | 9 | 2.8 | 25.0 |
| 3 | 0 | 5 | 0 | 13.9 |
| 4 | 0 | 6 | 0 | 16.7 |
| 5 | 0 | 7 | 0 | 19.4 |
| Mean | 0.4 | 6.8 | 1.1 | 18.8 |

*Experiment 2.*—This experiment was designed to eliminate many of the variants which unavoidably occurred in Experiment 1. Unfortunately it was necessary to compromise between transplanting roots at a time most suitable for seed production purposes when healthy control plots would most certainly become infected after establishment, or transplanting the roots at a less favourable time and covering them with cages until the vector disappeared in early summer. In order to attain the main objective of the experiment the latter procedure was adopted.

(i) *Method.*—On March 9, 1945, seed of the Chantenay variety was sown in each of eight three-rowed plots, in a uniform sandy loam soil at Burnley which had been treated, prior to sowing, with a complete fertilizer mixture applied at a rate equivalent to 9 cwt. per acre. These plots were arranged in two rows and were separated by paths two feet wide along their long and short axes. A pair of plots, one in each row, constituted a block. Each plot was completely covered with a muslin cage 4 ft. long, 3 ft. wide, and 18 in. high. These cages were provided with arm-holes to enable weeding, cultivating, and thinning to be performed without risk of infection from winged aphids (see Plate 4, Fig. 1).

On September 24, 1945, groups of infective *C. aegopodii* were introduced to single cages selected at random in each of the four blocks, and non-infective aphids to the four remaining cages. During October the vector multiplied rapidly and all plots became heavily infested. The plants exposed to viruliferous aphids developed typical virus symptoms and their foliage became stunted. The control plants supported a heavier infestation than the virus infected plants, without any apparent loss of vigour.

On November 8, 1945, the cages were sprayed thoroughly with a nicotine sulphate solution (1 in 400) and, on the following day, the roots were pulled, topped, and dipped in nicotine solution to destroy any aphids which might have survived the spraying.

Twelve evenly matched roots, free from growth cracks and blemishes, were then selected from one plot and used as a standard for selection from the remaining plots. The total weight of the standard group was 790 g. and, in order to qualify, each group of uniformly sized roots was required to be within 10 g. of this weight. Two hours after dipping, these groups, comprising individual replicates, were each planted in double-rowed plots in an adjacent area, and in positions corresponding to their locations in the original randomization. The roots were planted vertically every 12 in., in rows 24 in. apart, and covered to the bases of the petioles with compacted soil. Each root was then enclosed in a cylindrical muslin-covered cage.

A subsidiary experiment was also commenced in an insect-free field cage. In this experiment an infected and a healthy root were planted in each of 28 porcelain pots containing acid washed sand. The pots were watered twice weekly with 250 ml. of a complete nutrient solution suitable for the growth of carrots.

The roots established in the field plot experienced ideal conditions during the ten days following transplanting, owing to the occurrence of heavy rain and a period of cool weather.

(ii) *Growth Rate.*—During the following month the roots in the field plot produced considerable foliage growth. The cages were removed on December 13, 1945, and the plants were sprayed with a proprietary DDT emulsion every few days until, with the occurrence of normal summer temperatures, the danger of winged aphids passed.

On December 15, 1945, the height of every plant in the field plot was measured. As the plants had not formed seed stalks at that stage, measurements were obtained by bunching the leaves together in one hand, whilst recording the height of the tallest leaves to the nearest inch. A statistical analysis of these measurements (see Table 7), showed that initial differences in vigour, which were highly significant, occurred between the healthy and virus infected seed plants. Growth differences were even more apparent between the healthy and infected roots grown in sand culture, but as a number of plants in this trial had produced seed stalks, foliage measurements could not be obtained.

(iii) *Incidence of Root-Rot.*—During the period January to April 1946, the foliage of a number of the virus infected plants, in both experiments, wilted and died. Examination of the roots of these plants showed that a complete destruction of the cortical tissues had occurred, and in most cases only a shrivelled vascular cylinder remained. The numbers of plants which died in this manner before producing seed are recorded in Table 7.

TABLE 7

COMPARATIVE GROWTH AND SURVIVAL OF HEALTHY AND VIRUS INFECTED SEED PLANTS

| | Foliage Height in Field Plot on 13.xii.45 (in.) | Mortality from Root Decay | |
|---|---|---|---|
| | | Field Experiment (%) | Sand Culture Experiment (%) |
| Virus infected | 6.3 | 37.5 | 64 |
| Healthy | 13.9 | 0 | 0 |

At the conclusion of the experiment, roots of all plants were individually examined. It was found that healthy roots had considerably increased in girth since transplanting, and had produced sizeable lateral roots, some of which had attained a diameter of one centimetre. The surviving infected roots presented a marked contrast, as they had not increased in girth and their lateral roots were of a fibrous nature.

### (*b*) *Effect of Virus Infection on Seed Yield*

Throughout the summer, autumn, and early winter, the field plot established in Experiment 2 was sprayed at weekly intervals with DDT emulsion, to combat Rutherglen bug (*Nysius vinitor*) and *H. foeniculi*, both serious pests of carrot seed crops in Victoria. Seed umbels were harvested as they matured and stored in paper bags in the laboratory.

Surviving plants in the infected plots were very stunted and chlorotic and the most severely affected plants only produced primary umbels. The healthy plots, on the other hand, were uniformly vigorous and all plants developed many secondary umbels in addition to primary umbels (see Plate 4, Fig. 2).

(i) *Seed-Cleaning Method.*—Umbels harvested individually from each replicate were air-dried for several months, and then hand stripped. These bulk samples were freed from awns, and infertile seed was largely destroyed by rubbing between a rubber-covered wooden block and a corrugated rubber mat. The resultant debris was removed by means of three British Standard sieves (8 mesh, 16 mesh, and 20 mesh to the inch, respectively) and the draught from an electric fan. The three sieves were placed together in the order mentioned and held immediately above a fan, the blades of which were inclined at a slight angle to the horizontal. Seed was then poured into the topmost sieve and agitated for several minutes. The coarse top sieve allowed the passage of all the seed, but removed most of the coarse stalk material; the middle sieve held back the largest seed, or seed from which awns had not been completely removed; the bottom sieve prevented the passage of the smallest seed and most of the fine debris was blown away by the draught from the fan. The contents of the middle sieve were then transferred to the fine mesh sieve, and carefully agitated above the fan. This final operation removed most of the remaining debris, including light infertile seed which had survived the rubbing process, and the resultant sample compared favourably in appearance with good commercial samples.

(ii) *Germination Tests and Seed Yield.*—Laboratory germination tests were conducted in triplicate with samples of 100 seeds from each replicate. The germination of most samples was unexpectedly low, and seed from infected plots had a higher test than seed from healthy plots. Seeds which failed to germinate usually produced a copious fungal growth which was identified (following pathogenicity tests) as *Macrosporium carotae*, Ell. & Langl. This fungus is of minor importance on carrots in Victoria, but has been observed attacking and partially defoliating mature crops of susceptible varieties grown under wet conditions.

The lower infection occurring in seed from virus infected plots would appear to be due to the fact that most of this seed was produced in primary umbels, which matured earlier than secondary umbels, and when the fungus was less active. The relatively high percentage of infected seed from control plots was rather surprising, as the foliage of the seed plants showed only slight infection during the growing season.

Germination of the seed was improved after treatment with a fungicidal dust containing 1 per cent. ethyl mercury phosphate (New Improved Semesan Jr.), but the fungus was not completely controlled by this treatment.

The results of the germination tests together with the mean yields of cleaned seed from healthy and virus infected plots are recorded in Table 8.

The differences in seed yield from healthy and infected plots were highly significant.

TABLE 8

COMPARATIVE SEED YIELDS OF HEALTHY AND VIRUS INFECTED CARROTS

| | Yield of Cleaned Seed (Mean of 4 Replicates) | Mean Germination | |
|---|---|---|---|
| | | Untreated Seed | Treated N.I. Semesan Jr. |
| | (g.) | (%) | (%) |
| Healthy | 124.5 | 36.5 | 51.0 |
| Virus infected | 16.7 | 44.0 | 58.0 |

### (c) *Factors Associated with Root Decay*

While the above experiment was in progress an attempt was made to elucidate the factors responsible for root decay in seed plants.

The author was unable to find any record of a similar problem in the virus literature, but investigations by Kristoffersen (1921) concerned with the resistance of carrot varieties to winter storage rots, suggested a promising line of approach to the problem. Kristoffersen claimed that a positive correlation existed between low invert sugar content and rot resistance of stored roots, and that the correlation was more marked if the relative percentage of invert sugar to total sugar was considered, instead of the total percentage of invert sugar. A relative percentage of invert sugar to total sugar in excess of 45-50 per cent. indicated that the roots would rot in winter storage and it was also suggested that invert sugar might provide a better medium than cane sugar for the growth of parasites associated with root decay.

(i) *Root Analyses.*—In pursuance of this line of investigation samples of roots were selected for analysis from each of the muslin covered plots in Experiment 2. These samples were selected at random when the plots were harvested, and each replicate was analysed for reducing sugar and non-reducing sugars.

The results of the analyses are summarized in Table 9.

The above analytical determinations showed that the invert sugar content of virus infected roots was lower than that of healthy roots and that Kristoffersen's hypothesis did not explain the susceptibility of the former to root decay.

TABLE 9
SUGAR CONTENT OF INFECTED AND HEALTHY CHANTENAY CARROT ROOTS

| Sample | Reducing Sugars (as Invert) | | | Non-Reducing Sugars (as Sucrose) | | |
|---|---|---|---|---|---|---|
| | As Received | On Dry Basis | | As Received | On Dry Basis | |
| | (%) | (%) | (Angle) | (%) | (%) | (Angle)* |
| Healthy roots | 3.88 | 38.4 | 38.28° | 1.23 | 11.9 | 20.16° |
| Infected roots | 3.50 | 32.5 | 34.69° | 1.72 | 16.1 | 23.64° |
| Difference for significance 1% level | | | 3.38° | | | — |
| Difference for significance 5% level | | | — | | | 2.34° |

* Percentage figures have been transformed to angles (= arcsine √ percentage) as described by Snedecor 1940, and examined statistically by the analysis of variance method (Fisher and Wishart 1930).

Similarly, the relative percentage of invert sugar to total sugar was lower for infected roots (67 per cent.) than for healthy roots (76 per cent.) and, according to Kristoffersen, both would have rotted in winter storage, the latter being most susceptible. Although storage tests were not conducted, it is considered that such an assumption would have been illogical, in view of the performance of healthy and infected roots in the seed-production trial.

(ii) *Root Inoculations with Fungal Organisms.*—In order to determine whether virus infected carrot roots provided a better medium for fungal growth than healthy roots, inoculation experiments were conducted with organisms found associated with root decay in carrot seed crops. The following organisms were used: *Alternaria radicina* (M., Dr., & E.); *Sclerotium* sp.; *Fusarium* spp. (4); and *Verticillium* sp.

Needle prick inoculations with each organism were replicated three times on separate infected and healthy roots, which were then suspended in pairs over a free water surface in glass refrigerator jars, and incubated at 25°C.

Observations over a period of thirty days showed that root decay produced by the various organisms proceeded at a similar rate on both infected and healthy roots. The organisms were all weak parasites, or saprophytes, with the exception of *A. radicina* and *Sclerotium* sp., neither of which was associated with Victorian seed-crop failures. The species of Fusaria tested were the commonest organisms isolated from roots in early stages of decay during the investigation.

These results suggest that undue significance has been attached to the apparent biological breakdown of roots in carrot seed crops and that this phenomenon is a secondary development not responsible for the death of seed plants. It is postulated that the breakdown of infected transplanted carrot roots is a form of necrotic collapse resulting from virus infection, which may be accelerated by transplanting, but which does occur in plants which have not been transplanted.

The factors associated with the development of this phase of the disease are imperfectly understood, but it is believed that necrotic symptoms do not appear in carrot roots until plants are approaching maturity. All cultivated varieties of *D. carota* vary considerably, and it is thought that individual plants in a susceptible variety vary in their reactions to the virus, thus accounting for the fact that some seed plants do not develop root decay. In more susceptible species, like slender celery and coriander, necrotic symptoms usually occur, and very few plants survive the disease.

Environmental conditions also appear to be of importance in determing the mortality rate in seed crops grown from infected roots. The incidence of root-rot was higher in the above sand culture experiment than in the field trial (Experiment 2), yet the roots were obtained from the same source and lifted at the same time. In the former experiment, however, the plants were subjected to higher temperatures in a field cage, and grown in sand with low water-holding capacity. In seed crops dependent upon natural rainfall, the poorly developed lateral root systems of infected plants would be unable to supply their water requirements during dry periods, or to withstand the considerable leverage imposed by foliage movements in windy weather.

## XV. Control

### (*a*) *Regulation of Time of Sowing*

Virus free carrots can be produced in Victorian localities where natural rainfall or irrigation facilities permit the establishment of summer sown crops. In some districts late November sowings will remain healthy, whereas, in others, carrots should not be sown before mid-December.

Rigid sowing dates cannot be recommended for any one locality as the occurrence of late spring rains in an abnormal season may prolong the aphid infestation well into December, or even until early January. However, if such conditions do occur, aphid control measures should be regularly applied until the advent of normal summer temperatures prevent further multiplication of the vector.

### (*b*) *Virus Control by Combating the Vector*

The few successful attempts "to control virus diseases in the field by combating the vectors" are summarized by Bawden (1943), who also suggests that such measures may be practical in small scale intensively cultivated crops. All successful vector control experiments have depended upon regular applications of insecticidal sprays or dusts during the period when the vectors were active. In preliminary experiments conducted during 1943 at a Dandenong market-garden, nicotine sprays and dusts applied to spring sown carrots at weekly intervals failed to control the disease.

During 1945-46 a further experiment was conducted at Burnley to test the efficacy of the then new insecticides DDT (dichlordiphenyltrichlorethane) and 666 (hexachlorcyclohexane) in controlling *C. aegopodii.*

(i) *Method.*—On September 25, 1945, an experimental area was sown with carrots (var. Chantenay) in rows one foot apart.

The following spray treatments, which were applied at approximately weekly intervals, were replicated four times in randomized blocks. Plot units consisted of four parallel rows fifteen feet long.

DDT. An aqueous emulsion containing 0.1 per cent. DDT in aromatic solvent (prepared from an emulsion concentrate containing 20 per cent. para para isomer of DDT).*

666. An aqueous emulsion containing 0.1 per cent. hexachlorcyclohexane in aromatic solvent (prepared from an emulsion concentrate containing 1.9 per cent. of gamma isomer of hexachlorcyclohexane).*

Nicotine sulphate (40 per cent. nicotine) 1/600 + white oil emulsion 1/150.

Control plots were not sprayed.

The above insecticides were applied by means of a knapsack, hand operated spray pump. Spraying was commenced on October 12, 1945, and concluded on December 15, 1945—a total of eight applications.

At the appropriate time the central rows of each plot were thinned to fifty plants per row. The outer buffer rows were not thinned with the same degree of accuracy.

The experimental plot received normal culture treatment and was watered by overhead sprinklers. Watering was not required during the spraying period.

(ii) *Aphid Control.*—Observations on November 9, 1945, showed that the control plots were heavily infested with *C. aegopodii;* a few aphids were present on 666 and nicotine sulphate-white oil plots, but aphids could not be found on DDT plots. A heavy infestation persisted on the unsprayed plots until the occurrence of high temperature conditions in mid-December.

On the day preceding the final spray application aphid counts were obtained on samples of twenty-five leaflets selected at random from the central rows of each replicate.** The results of these counts, which are recorded in Table 10, confirmed the earlier observations that DDT was more effective than 666 and nicotine sulphate-white oil in controlling *C. aegopodii.*

TABLE 10

POPULATIONS OF C. AEGOPODII ON SPRAYED AND UNSPRAYED CARROT FOLIAGE

| Treatment | Mean Numbers of Aphids on Samples of 25 Leaflets | |
|---|---|---|
| | Alate | Apterae |
| DDT | 1.25 | 0 |
| 666 | 0.5 | 2 |
| Nicotine sulphate | 3.75 | 4 |
| Control | 13.0 | 1469 |

At an early stage in the experiment, differences in foliage vigour between the sprayed and unsprayed plots became apparent, and by mid-November the DDT replicates were more vigorous than those of the other treatments and had produced foliage at least twice the height of that of the unsprayed plots. Foliage

* Products of Imperial Chemical Industries of Aust. & N.Z. Ltd.

** Aphid counts were made by Mr. T. W. Hogan, Senior Entomologist, Department of Agriculture, Victoria.

dwarfing in the control plots resulted from the combined effect of virus infection and aphid feeding injury, but the former was apparently most important, as only slight recovery occurred when the aphids disappeared.

(iii) *Virus Control.*—On February 25, 1946, the numbers of virus infected plants in the central rows of each replicate were recorded, and the results (see Table 11) subjected to an analysis of variance.

TABLE 11

EFFECT OF SPRAY TREATMENT ON VIRUS INCIDENCE

| Treatment | Virus Infected Plants on 25.ii.46 (%) | (Angle) |
|---|---|---|
| DDT | 68.2 | 55.97° |
| 666 | 86.5 | 70.07° |
| Nicotine sulphate | 88.7 | 71.06° |
| Control | 100 | 90.00° |
| Difference for significance 1% level | | 18.95° |
| Difference for significance 5% level | | 13.18° |

It is of interest to note that the order of effectiveness of the three insecticides was accurately forecast by the vector population counts recorded in Table 10.

(iv) *Yield.*—On April 9, 1946, roots from the centre rows of each plot were pulled, graded, and weighed. Grading was carried out according to the following standards which were adopted for carrots grown under contract to the Commonwealth during the last war: "To be sound, clean, of minimum diameter 1½ in. and maximum 4 in., with a minimum length of 4 in. when the diameter is between 1½ in. and 2 in. . . . fresh (not withered), reasonably free from growth cracks, excessive rootlets and woodiness, and free from forking and all damage including damage resulting from disease and insect pests. Mis-shapen or malformed roots, or roots producing seed heads prior to harvesting not to be included." The results of this experiment, which were subjected to an analysis of variance, are recorded in Table 12.

TABLE 12

EFFECT OF SPRAY TREATMENTS ON YIELD OF CARROTS

| Treatment | Total Wt. (lb.) | Marketable Wt. (lb.) | Unmarketable Wt. (lb.) | | |
|---|---|---|---|---|---|
| | | | Undersized | Split | Rotted |
| DDT | 17.7 | 8.6 | 2.9 | 6.2 | 2.4 |
| 666 | 14.3 | 5.2 | 4.4 | 4.7 | 2.2 |
| Nicotine sulphate | 12.8 | 4.6 | 3.6 | 4.6 | 2.4 |
| Control | 9.0 | 1.3 | 4.3 | 3.5 | 0.8 |
| Difference for significance 1% level | 7.4 | 6.0 | — | — | — |
| Difference for significance 5% level | 5.1 | 4.2 | — | — | — |

In comparing the results obtained from the various treatments with the control, DDT alone produced highly significant increases in total yield and yield of marketable roots, the latter being more than a sixfold increase. The total yield of roots was also significantly increased (5 per cent. level) by 666.

The amount of virus infection in the DDT and 666 plots was significantly less (1 per cent. level) than in the untreated plots and disease reduction was significantly greater (5 per cent. level) from DDT.

While the Burnley experiment was in progress, Taylor (1945) reported the results of spraying trials against *C. aegopodii* on carrots in New Zealand.* In these trials an aqueous suspension of DDT gave less effective control than 666 and nicotine sulphate, but the results of the two experiments are not comparable, as a soluble form of DDT was used in the Burnley experiment.

In a recent report from the United States, Pound and Chapman (1947) recorded control of the Aster Yellows virus in carrots by controlling its vector, *Macrosteles divisus*. In this experiment six applications of a wettable DDT spray, at ten day intervals, increased the yield of carrots by four tons above the unsprayed control and decreased virus infection by 38 per cent.

The author has been unable to find any further records of the control of virus diseases by means of DDT, but it is anticipated that practical field control of other insect transmitted viruses will be demonstrated in the future, as the residual effect of this insecticide overcomes one of the greatest disadvantages of older insecticides which have been used unsuccessfully in many vector control experiments.

### (*c*) *Disease Control by the use of Virus Tolerant Varieties*

In the course of field varietal trials the existence of degrees of "resistance" became apparent between the various virus tolerant varieties and, in order to evaluate the resistance of the most promising varieties, a yield trial was sown at Burnley on October 4, 1945.

(i) *Method.*—In this trial, a commercial line of seed of the susceptible Chantenay variety was chosen as a standard for comparison with the following virus tolerant selections:

(1) Champion Intermediate (syn. Osborne Park), from Balcatta, Western Australia.

(2) Imperator X (stated to be the result of a cross between Imperator and Champion Intermediate), from Balcatta, Western Australia.

(3) Victorian strain (selected, at Burnley, from a virus tolerant strain of unknown origin, obtained from a metropolitan market-garden in 1942).

Each of the above varieties, and Chantenay, was replicated four times in randomized blocks. Individual plots consisted of six rows spaced one foot apart by twenty-five feet long, the two outer rows being regarded as buffers.

At thinning time a total of 150 evenly spaced plants was left in each plot, excluding the two buffer rows.

* The carrot virus disease has not been recorded in New Zealand.

All varieties became heavily infested with the vector shortly after emergence and 100 per cent. virus infection occurred. Aphid control measures were not applied.

The experiment was harvested on April 11 and 12, 1946, and the roots from individual plots were weighed immediately after lifting. The roots were graded as in the above vector control spraying experiment.

(ii) *Yield.*—The yield data obtained from the experiment were examined statistically. The relevant results are summarized in Table 13.

TABLE 13

COMPARATIVE YIELDS OF VIRUS TOLERANT AND VIRUS SUSCEPTIBLE CARROT VARIETIES

| Variety | Total Yield | Yield Marketable Roots | | | Red Core |
|---|---|---|---|---|---|
| | (lb.) | (lb.) | (%) | (Angle) | (%) |
| Victorian strain | 43.9 | 30.7 | 61.2 | 51.49° | 17 |
| Champion Intermediate | 35.0 | 17.2 | 37.5 | 37.73° | 52 |
| Imperator X | 34.6 | 14.4 | 28.3 | 31.98° | 41 |
| Chantenay | 21.3 | 8.2 | 21.2 | 27.21° | 96 |
| Difference for significance 1% level | 11.4 | 6.2 | | 6.44° | |
| Difference for significance 5% level | 7.9 | | | | |

In the above experiment it was unfortunate that a comparison could not be made between the yields of the four varieties in the absence of virus infection. However, the results showed that the Victorian strain which exhibited least foliage mottling and dwarfing also produced the highest yield and percentage of marketable roots, whereas Chantenay, the most severely affected variety, occupied the converse position. Moreover, Chantenay is generally regarded as being a high yielding variety, and it is, therefore, reasonable to assume that the yield differences were largely attributable to the virus.

Roots produced by the Victorian strain were of a fairly uniform type resembling, but slightly shorter and more stumped than, those of the Champion Intermediate variety. They were somewhat paler than those of the other varieties and were predominantly yellow cored. The roots pulled "cleanly" and lacked the unsightly lateral root depressions sometimes shown by the Champion Intermediate variety.

A hollow crown was more characteristic of this strain than of Champion Intermediate, and the junction of the petioles with the crown in the former, resembled that of quality varieties like Chantenay. The Champion Intermediate variety is prone to "neckiness" and "greening" in the crown region. Reference to Plate 4, Figure 3, will show some of the features which distinguish the two varieties.

In June 1947, approximately 10 cwt. of roots grown from the Burnley selected strain were planted, after further selection, at the Horticultural Research Station, Scoresby. The seed obtained from this crop will serve as a nucleus for

further agronomic improvement of the variety. The variety will ultimately be named and distributed to the seed trade.

## XVI. Discussion

Certain symptoms of the Australian carrot disease resemble those described for the American diseases caused by the Californian Aster Yellows (Severin 1932) and Western Celery Mosaic (Severin and Freitag 1938) viruses, but there seems to be little doubt that the local virus is distinct from either of these viruses.

The virus resembles Aster Yellows in being a persistent virus, but differs in its mode of transmission, and in its host range. It is unlikely, moreover, that the presence in Australia of Aster Yellows would have remained unnoticed, because it causes economically serious diseases in a number of completely unrelated host plants.

There appears to be little or no resemblance between the carrot virus and the Western Celery Mosaic virus. The latter, as the name suggests, infects celery, which is immune to the carrot virus, is mechanically transmissible, and is non-persistent in a number of aphid vectors which feed on celery.

The Australian disease appears to be unique in that it has a specific vector which is itself a serious pest of carrots, and this aphid occurs in very large numbers when environmental conditions are favourable, thus carrying the virus to every plant in infested crops.

## XVII. Acknowledgments

The work described in this paper was carried out as part of the research programme of the Biological Branch, Department of Agriculture, Victoria.

Grateful acknowledgment is made of the substantial aid rendered by Dr. B. J. Grieve who, firstly as a co-worker, and later with helpful advice, contributed largely to all phases of the investigation.

The author is also indebted to Mr. C. W. Corrigan of Dandenong for valuable assistance in field work; to Dr. F. W. Whitaker of the United States Department of Agriculture, Mr. M. E. Hutton, Division of Plant Industry, C.S.I.R., and Mr. James Arbuckle, Balcatta, Western Australia, who supplied seed of carrot varieties for resistance trials; and to officers of the Horticultural Division, Department of Agriculture, Victoria, who made possible much of the field survey work.

The analytical determinations were conducted by Mr. W. R. Jewell, Agricultural Research Chemist, Department of Agriculture, Victoria, and weed hosts were identified by Mr. A. W. Jessep, Government Botanist, National Herbarium, Melbourne.

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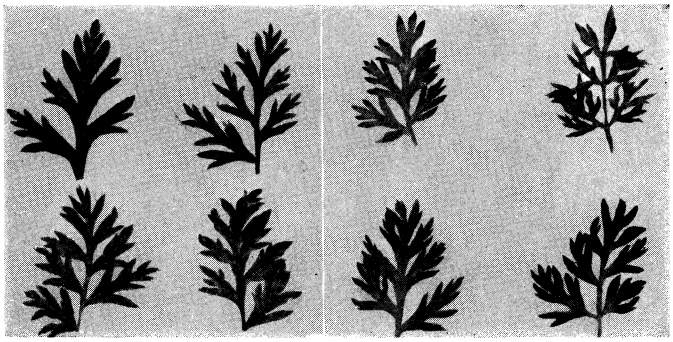

Fig. 1

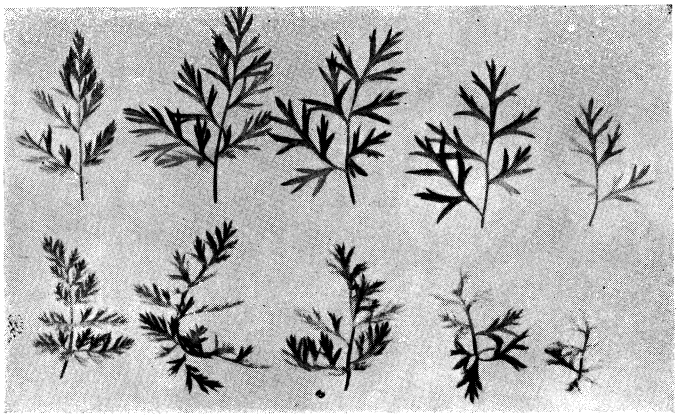

Fig. 2

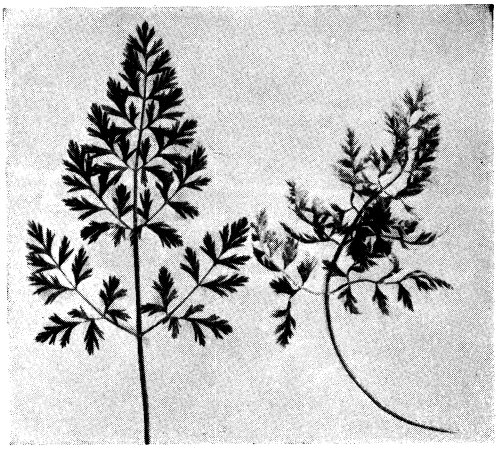

Fig. 3

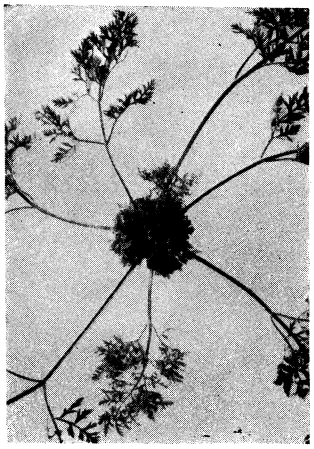

Fig. 4

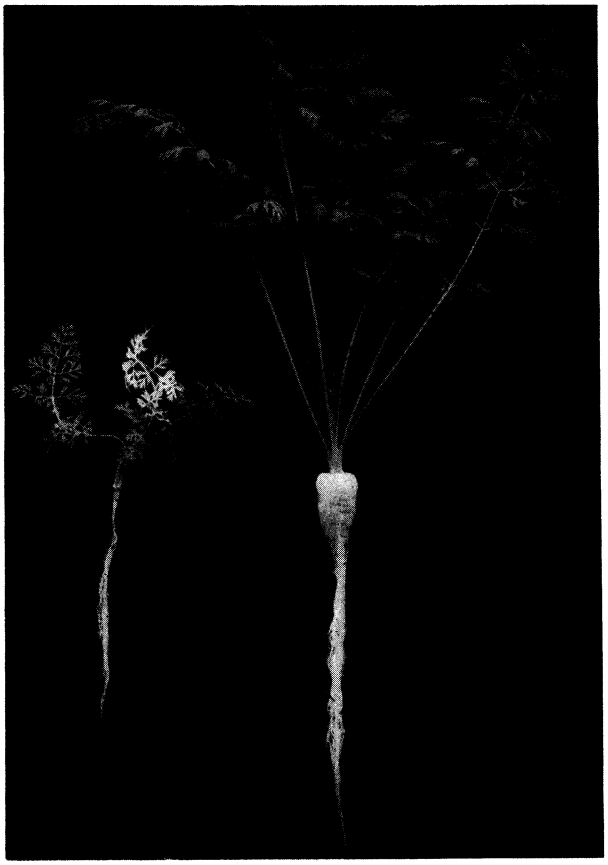

Fig. 1

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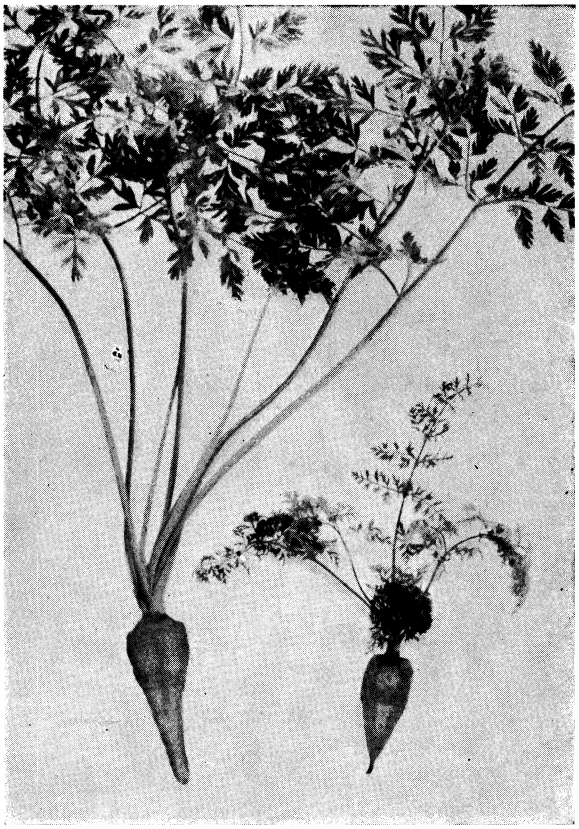

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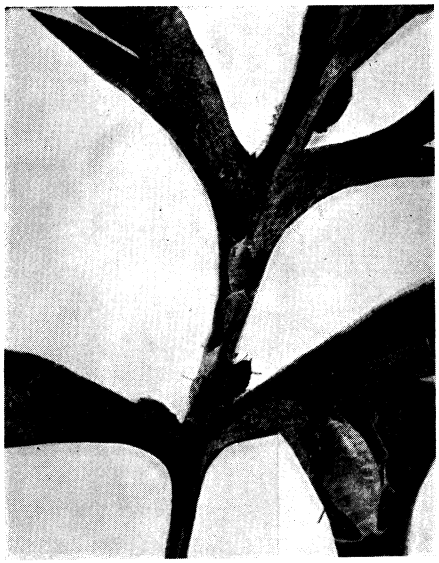


Fig. 2

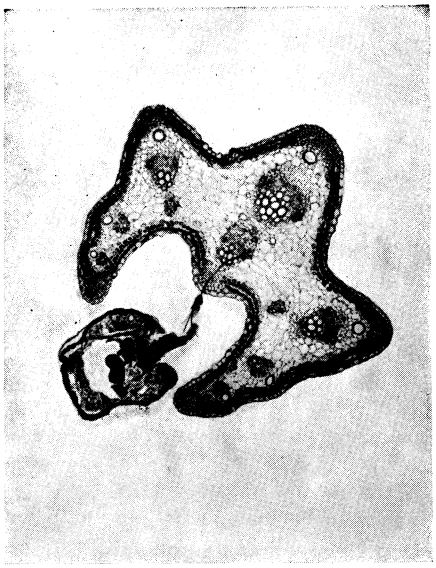
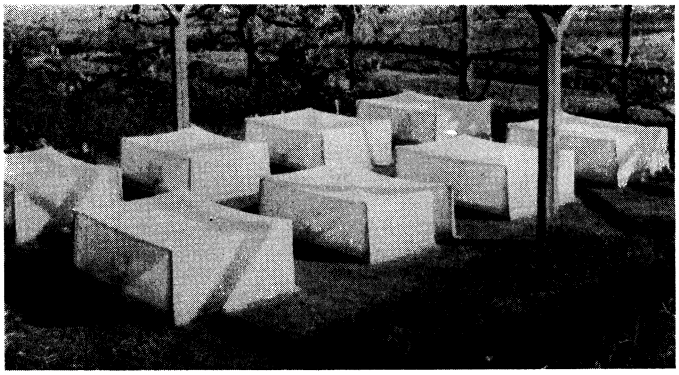


Fig. 3



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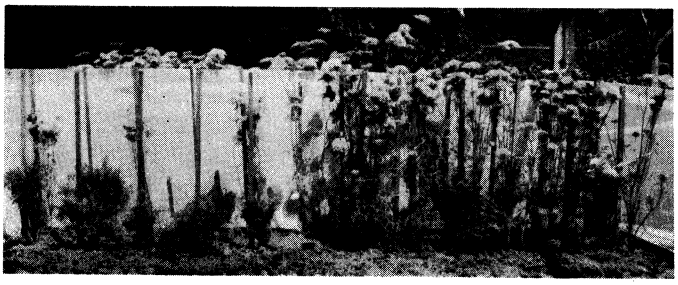

Fig. 2

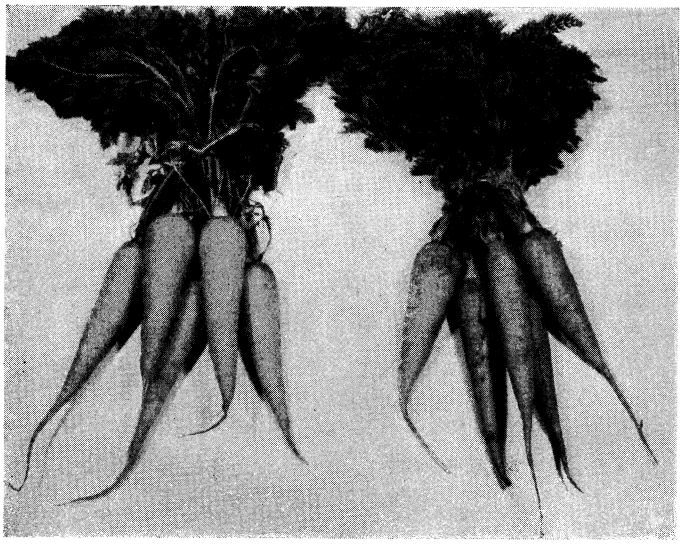

Fig. 3

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## EXPLANATION OF PLATES 1-4

### PLATE 1

Fig. 1.—Leaflets from infected carrots showing various stages of mosaic mottling and marginal reddening. The leaflet in the top left-hand corner is from a healthy plant.

Fig. 2.—Above, leaflets from a healthy carrot plant. Below, leaflets from an infected plant of the same age showing twisting of petioles and subpetioles, and progressive increase in intensity of mosaic mottling with age of leaves (from left to right).

Fig. 3.—Left, healthy carrot leaf. Right, infected leaf showing "S"-shaped bending of the petiole.

Fig. 4.—A recently infected mature plant showing rosetting of the youngest leaves.

PLATE 2

Fig. 1.—Left, dwarfed experimentally infected carrot plant. Right, healthy control plant of the same age.

Fig. 2.—Infected and healthy slender celery showing, left, petiole epinasty and recurving of younger leaves 21 days after inoculation with infective aphids.

PLATE 3

Fig. 1.—Transmission of the virus to carrot by core grafting, showing graft union. The plant on the left was grafted with a core from a healthy root and that on the right with a core from an infected root.

Fig. 2.—*Cavariella aegopodii* feeding on a carrot leaf. Inset, a single aphid. x 10.

Fig. 3.—Section of a carrot leaf petiole showing the stylet of *Cavariella aegopodii* entering the phloem tissue. x 38.

PLATE 4

Fig. 1.—Muslin covered plots in which healthy and infected carrot roots were grown for seed production experiments.

Fig. 2.—Carrot seed plants grown from infected (left) and healthy (right) transplanted roots.

Fig. 3.—Typical specimens of two virus tolerant carrot varieties of local origin. Left, Burnley selection of a Victorian strain; right, a Western Australian variety, Champion Intermediate.