STUDIES ON *Oliarus atkinsoni* Myers (HEMIPTERA : CIXIIDAE), VECTOR OF THE "YELLOW-LEAF" DISEASE OF *Phormium tenax* Forst.

IV.—DISEASE-VECTOR RELATIONSHIPS

By R. A. CUMBER, Entomological Research Station, Department of Scientific and Industrial Research, Foxton

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Summary

Experiments giving information on the disease-vector relationships of adult and nymphs of *O. atkinsoni* are described. It appears probable that the disease is induced by uymphal feeding. No evidence for bugs from healthy plants becoming infective after feeding on leaf of diseased plants, was obtained. The sudden die-back condition, and deaths, were common especially in experiments on nymphal transmission. The relative efficiency of adult caging techniques has been determined.

INTRODUCTION

It has been established that the yellow-leaf disease of *Phormium* can be induced in seedling material by adults of *O. atkinsoni* (Cumber, 1953a). Transmission by grafting and mechanical means (Boyce *et al.*, 1953) has been taken as proof that the pathogen concerned is a virus. The experiments described in the present account were designed to obtain further information on the virus-vector relationships which have not hitherto been studied in the family Cixiidae. In addition, the intended use of the bug as an infecting agent to detect seedlings resistant to the disease called for an efficient technique.

The sudden die-back condition (Cumber, 1953b) appeared in most experiments, and this, together with the number of deaths, is recorded.

The term "infection feed" refers to the feeding upon a plant (usually diseased) for the purpose of infecting the bug, whereas "test feed" refers to the feeding upon a plant (usually healthy) to test the effect of the bug.

In all experiments, seedlings of the variety "301" were used. Control plants were given the same caging treatments as test plants, and when the setting up of the experiment extended over several days controls were set up as the trial proceeded.

ADULT TRANSMISSION

Eleven experiments were carried out. Seedlings 7 to 8 months old were used except where indicated otherwise. These were field-raised but had been removed from beds before adults of the known vector were present. Test feeding was carried out as in Fig. 1 except where older established plants were used. Damp cotton-wool kept the roots moist, and a loose dry plug held the bugs within the tubes which were laid on their sides. Infection feeding was carried out as in Fig. 2 on pot plants from the 1950–51 trials. The bugs were collected by a uniform technique from five separated areas, their source, for the purpose of comparing experiments, being indicated in each case. (Areas 1, 2, 3, 4, and 6, diseased; area 5, healthy.)

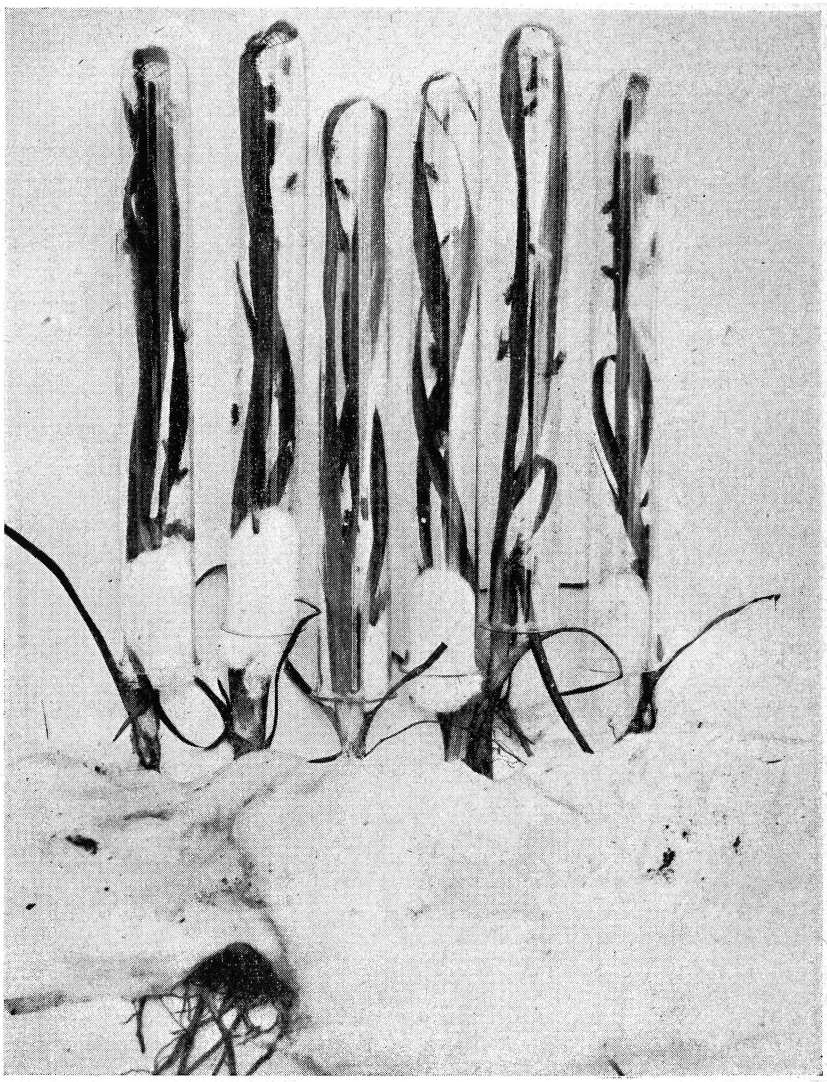

FIG. 1.—Method of test-feeding in the majority of experiments.

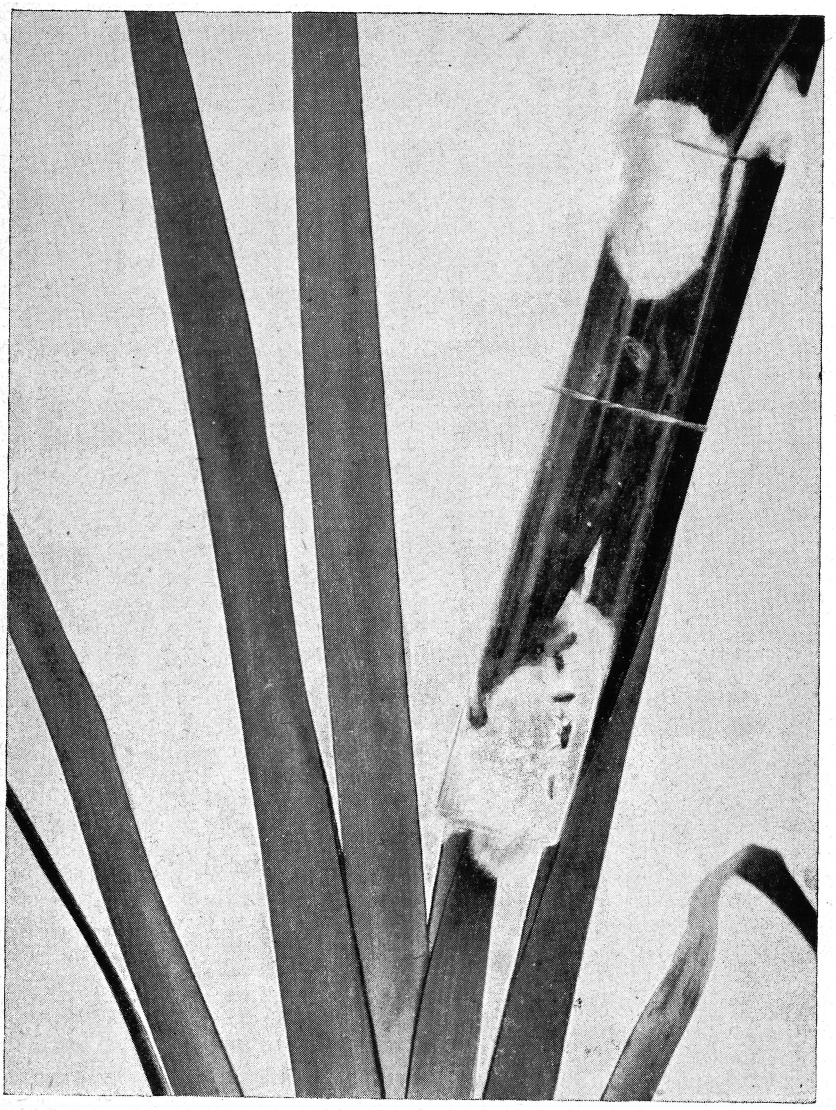

FIG. 2.—Method of infection-feeding in experiments.

Experiment 1.—True feeding effect: set up 27/11/51–7/12/51, analysed 1/7/53.

This experiment was set up to determine whether bugs affected plants through feeding only.

Bugs from apparently healthy bushes (area 5) were caged on seedlings after a 24-hr starvation period. They were removed after 10 days, and seedlings were transferred to 6 in. pots and held in the insectary. The total length (inches) of leaf showing no yellowing (main fan only) was measured on 5/5/52 and 1/7/53. The numbers of bugs used and results obtained are shown in Table 1.

TABLE 1.—True Feeding Effect.

No. of bugs per plant	No. of plants	Average length (in.) of leaf showing no yellowing*		Plants	
		5/5/52	1/7/53	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
1	15	62 (15)	160 (14)	0	1
2	15	64 (15)	155 (14)	0	2
4	15	62 (15)	170 (14)	0	2
8	15	58 (15)	174 (13)	1†	2
16	15	47 (13)	157 (13)	0	0
Controls no bugs	30	53 (29)	159 (29)	0	0

*Number of plants alive at assessment indicated in brackets.
†Not included in measurements.

There is no indication of lasting injury to growth from feeding effects.

Experiment 2.—Persistence of infective capacity from nymph to adult: set up 15–23/11/51, analysed 26/5/53.

Nymphs were collected from the bases of diseased bushes (area 3). Adults emerging in captivity in the absence of food and within 3 to 4 days of their collection as nymphs were test fed. Observations showed that no feeding takes place for 3 to 4 days from the commencement of the final moult. Nymphs were held on moist filter papers in petri dishes during adult emergence. Five bugs per plant were used, and the test feed period comprised 10 days, after which seedlings were transferred to 6 in. pots held in the insectary. Results are shown in Table 2.

TABLE 2.—Persistence of Infectivity from Nymph to Adult

	No. of plants	No. of bugs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Test	25	5	2	0	3
Control	26	0	3	0	5

No evidence that adults became infective through nymphal feeding was obtained.

Experiment 3.—Relative efficiency of males and females in transmitting the disease: set up 30/11/51–3/12/51, analysed 26/5/53.

Bugs were collected from four diseased areas. Five bugs per plant were caged as in Fig. 1, but with 4 in. by $\frac{3}{4}$ in. glass tubes. After a 7-day test feed, seedlings were transferred to 6 in. pots and held in the insectary. The sexes are readily separable on account of the larger size and presence of abdominal mealy pads in the female, etc. The results are summarized in Table 3.

TABLE 3.—Relative Efficiency of Males and Females.

	Replications of 10 plants						
Bugs used	Area 1	Area 2	Area 3	Area 4	Area 4	Area 4	Totals
Males	1·1·1*	0·1·1	2·0·1	1·2·0	0·1·1	0·0·1	4·5·5
Females	0·0·3	0·0·0	2·2·0	1·1·0	2·1·1	0·1·1	5·5·5
Controls no bugs	0·0·3	0·0·0	0·0·0	0·0·0	0·0·1	0·0·0	0·0·4

*Number of dead plants, plants with typical *Oliarus*-induced symptoms, and plants with sudden die-back symptoms are shown in that order.

The results do not indicate that there is any difference in the efficiency with which males and females transmit the disease. There was a marked difference in survival of test and control plants.

Experiment 4.—Ability of adults from diseased and non-diseased bushes to transmit the disease: set up 26–28/11/51, analysed 26/5/53.

The healthy (area 5) and diseased (area 3) bushes were separated by less than 150 yards. Since the experiment commenced early in the flight season, migration should have been reduced to a minimum. The proximity of collection sites made it unlikely that two races were concerned.

The experiment was set up in two parts, the bugs being: (a) caged by means of test tubes with cotton-wool stoppers on middle-aged leaves of 18-month-old seedlings established in 6 in. pots, and (b) caged as in Fig. 1 but using 4 in. by $\frac{3}{4}$ in. glass tubes and 7- to 8-month-old seedlings, the plants being transferred to 6 in. pots and, like (a), held in the insectary. Five bugs per plant were used and 7-day test feeds given. The results given in Table 4 are strong evidence against a toxin hypothesis, although it would be necessary to render one half of a sample of bugs infective through infection feeding to preclude the possibility of toxic races.

TABLE 4.—Effect of Host Plant on Ability to Transmit.*

	No. of plants	No. of bugs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Bugs from diseased plants	25 (15)	5	0 (0)	4 (1)	2 (2)
Bugs from healthy plants	25 (15)	5	0 (0)	0 (0)	2 (3)
Controls (no bugs)	25 (15)	5	0 (0)	0 (0)	2 (2)

*Results of part "b" are given in brackets.

Experiment 5 (a).—Effect of infection feeding upon infective capacity: set up 19–30/11/51, analysed 8/6/53.

Fifth-instar nymphs collected from healthy bushes (area 5) were placed together with dead basal material (carefully checked to ensure that no living root or leaf was included) in drums covered with wire gauze. Emerging adults were infection fed for 2 to 3 days on the second youngest leaf of healthy or diseased pot plants. Plants receiving bugs which had not fed as adults, in addition to control plants receiving no bugs, were set up. Five bugs per plant were used, and 10-day test feeds given, after which plants were transferred to 6 in. pots held in the insectary. Results are shown in Table 5 (unbracketed numbers).

Experiment 5 (b).—Effect of infection feeding upon infective capacity: set up 27/11/52–8/12/52, analysed 11/6/53.

This experiment differs from the preceding one in that the bugs used were collected as adults from apparently healthy bushes (area 5). Two-day infection and 9-day test feeds were given and 10 bugs per plant were used. Results are shown by the bracketed numbers in Table 5.

TABLE 5.—Effect of Infection Feeding upon Infective Capacity.

	No. of plants	No. of bugs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Bugs fed on diseased plants	15 (20)	5 (10)	2 (0)	0 (0)	5 (8)
Bugs fed on healthy plants	15 (20)	5 (10)	0 (1)	0 (2)	3 (5)
Bugs unfed as adults*	15 (20)	5 (10)	1 (1)	0 (0)	2 (2)
Controls (no bugs)	15 (20)	—	0 (0)	0 (0)	6 (2)

*Read "Field bugs directly to test plants" in case of bracketed figures of experiment 5 (b).

These experiments provide no evidence for adult bugs being rendered infective by feeding upon leaf of diseased pot plants.

The two positive infections are unexplained. The increase in number of plants showing sudden die-back symptoms where bugs have fed

on diseased plants is of interest, but in view of the high number in the controls, it is of doubtful significance.

Experiment 6.—Effect of test feeding time on infective capacity of adults: set up 20–26/11/51, analysed 24/6/53.

Bugs were collected from diseased bushes (area 4) and starved 24 hours before being tested. Ten bugs per plant were used and plants were subsequently transferred to 6 in. pots and held in the insectary. The test-feed times and results obtained are shown in Table 6.

TABLE 6.—Effect of Test-feeding Time on Infective Capacity.

Test-feed times	Replications of five plants						Totals
	1	2	3	4	5	6	
15 min.	0·0·1*	0·0·0	0·0·0	1·0·0	0·0·1	0·0·1	1·0·3
30 min.	2·0·1	0·0·0	0·0·1	0·0·0	0·0·0	1·1·1	3·1·3
1 hr	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0
3 hr	0·0·0	0·0·0	0·1·0	0·0·0	0·0·0	0·0·0	0·1·0
6 hr	0·0·0	0·0·1	0·1·2	0·0·0	0·1·0	0·0·0	0·2·3
1 day	0·0·0	0·0·0	1·0·0	0·1·0	0·0·0	0·0·1	1·1·1
3 days	0·1·0	0·1·0	0·0·0	0·2·0	0·0·0	0·0·0	0·4·0
6 days	0·1·0	0·0·0	0·0·1	0·0·0	1·1·1	1·1·0	2·3·2
Controls (i)	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0
Controls (ii)	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·1	0·0·1

*Number of dead plants, plants with typical *Oliarus*-induced symptoms, and plants with sudden die-back symptoms, are shown in that order.

Although the number of positive infections is small, there is an increase (except in one case) where the duration of the test feed is 6 hr. or more.

Experiment 7.—Effect of infection feeding time upon infective capacity: set up 29/11–21/12/51, analysed 17/6/53.

Bugs were collected from healthy bushes (area 5) and starved for 24 hr. in darkness before being given infection feeds. The starvation caused the majority of the bugs to commence probing within a few seconds of being placed on plants. Five bugs per plant were used and 10-day test feeds in 4 in. by $\frac{3}{4}$ in. glass tubes were given.

Plants were then transferred to 6 in. pots and held in the insectary. Additional controls at the end of the experiment were set up to determine any changes which might have occurred in the field sampling area during the setting up of the trial. Details and results are shown in Table 7.

TABLE 7.—Effect of Infection Feeding Time upon Infective Capacity.
(Replications of six plants were used except where indicated otherwise.)

Duration of infection feed	10 min.	20 min.	2 hr	4 hr	6 hr	1 day	2 days	4 days
Bugs fed on— Infected plant No. 1	0·0·3*	0·0·1	0·0·0	0·0·0	0·0·0	0·0·0	1·0·0	0·0·0 (5 plants)
Infected plant No. 2	0·0·1	0·1·0	0·0·0	0·1·0	0·1·1	0·0·0	0·0·0	0·0·0 (4 plants)
Infected plant No. 3	0·0·2	0·0·2	0·0·1	0·0·1	0·0·0	0·0·0	0·0·1	0·0·0 (3 plants)
Infected plant No. 4	0·0·3	0·0·1	0·0·0	0·0·0	0·0·0	0·0·0	0·0·1	0·0·0 (2 plants)
Infected plant No. 5	0·1·1	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0	0·0·0 (7 plants)
Infected plant No. 6	0·0·0	0·0·1	0·0·0	0·0·0	0·0·1	0·0·0	0·0·0	0·0·2
Controls (no bugs)	0·1·1	1·0·0	0·0·1	0·0·0	0·0·0	2·0·0	0·0·1	0·0·0
Field bugs directly to plants	0·0·1	0·0·2	0·0·0	0·0·1	0·0·1	0·0·0	1·1·1	2·0·1 (20 plants)

*The three readings refer to plants which died during experiment; plants with typical *Oliarus*-induced symptoms; plants with sudden die-back symptoms, in that order.

The experiment does little more than verify the results of Experiments 5 (a), (b), which do not indicate that infection feeding of apparently non-infected adult bugs increases their infectivity under the conditions of experiment. The presence of a plant with typical *Oliarus*-induced symptoms amongst those receiving no bugs is unexplained. The numbers of infections do not vary noticeably in the test and control groups, and the same may be said for the numbers of plants showing sudden die-back symptoms. It is perhaps of some interest that 3 of the 4 positive test seedlings occur in groups fed on the one infected plant (No. 2).

Experiment 8.—Effect of test feeding site on infective capacity: set up 8/1/52, analysed 30/6/53.

Bugs were collected from diseased bushes in area 3. Ten bugs per plant were test-fed for 7 days using 4 in. by $\frac{3}{4}$ in. glass tubes. In the case of root feeding, some roots had access to moisture. Bugs were not starved and, following treatments, plants were transferred to seed-boxes (10 plants per box) and held in the insectary. Details and results are shown in Table 8.

TABLE 8.—Effect of Test-feeding Site on Infective Capacity.

Site of test feed	No. of plants	Plants		
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Second youngest leaf	30	3	2	2
Third youngest leaf	30	2	0	3
All leaves	30	10	0	0
Some roots	30	5	0	7
Controls (no bugs)	30	1	0	3

There is some evidence for increased transmitting ability where feeding is on the second youngest leaf. The large number of plants with sudden die-back symptoms in root feedings, and the large number of deaths in this group and that in which test feeding was on all leaves, are noted.

Experiment 9.—Ability of a single bug to infect a succession of plants: set up 18–20/12/51, analysed 6/7/53.

Bugs from diseased bushes (area 4) were starved for 24 hr. then given 20 min. test feeds on 3 successive plants, there being a rest period of 20 min. between feeds. After a further 24 hr. starvation 2 more test feeds were given as previously. Following a further 24 hr.

starvation, bugs remaining alive were given a 4 hr. test feed. Fifty bugs were tested, 18 feeding on 3, 27 on 5, and 5 on 6 successive plants, and controls numbered 50. Owing to restricted insectary space, seedlings were subsequently planted out in a shade house thus incurring some risk of infection from the field.

Final analysis revealed 44 dead plants and 1 with typical *Oliarus*-induced symptoms amongst the test plants; no dead plants and three with typical *Oliarus*-induced symptoms in the controls. Thus no evidence was obtained for the ability of a single bug to infect a succession of plants. Dead plants showed a regional tendency which may have borne some relationship to position in the shade-house.

Experiment 10.—Persistence of virus in vector: set up 23–28/1/52, analysed 3/7/53.

Adult bugs were collected from diseased bushes (area 3) and during starvation periods were held in the dark in tubes surrounded by moist cotton-wool, this treatment being designed to reduce activity. Five bugs per plant were used, and the test feed lasted 3 days. After treatments seedlings were planted out in a shade-house. Details of the starvation period and results are shown in Table 9.

TABLE 9.—Persistence of Virus in Vector.

Treatment of bugs	No. of plants	Plants		
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Controls (no bugs)	30	0	0	0
Bugs direct to plants	30	1	0	0
Starvation—2 days	30	4	0	0
Starvation—5 days	25	1	0	0

No positive infections were obtained, nor were sudden die-back symptoms found.

Experiment 11.—Rate of travel of virus from point of insertion: set up 24–29/1/52, analysed 3/7/53.

Bugs from diseased bushes (area 1) were caged by means of test tubes on the 3rd youngest leaf of 19-month old seedlings well established in boxes of 12 and held in the shade-house. Eight bugs per plant, starved for 24 hr. prior to test feeding, were used. After feeding, the leaf was severed 2 in. below the lowest point at which feeding was possible. Details and results are shown in Table 10.

TABLE 10.—Rate of Travel of Virus .

Treatment of bugs	No. of plants	Plants		
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Test feed—1 hour	12	0	0	1
Test feed—24 hours	12	1	1	3
Test feed—4 days	12	2	0	1
Controls (no bugs) (i)	12	7	0	4
Controls (no bugs) (ii)	12	1	0	4

Since only one typical *Oliarus*-induced infection was obtained and there was a possibility of field infection, conclusions cannot be drawn.

Experiments to determine the existence of a latent period in the vector were unsuccessful.

SUMMARY OF RESULTS FROM ADULT TRANSMISSION EXPERIMENTS

The results obtained in these experiments may be summarized as follows:—No reduction in the growth rates of plants receiving moderate numbers of bugs from healthy bushes, were detected. There was no indication that adults become infective as a result of nymphal feeding on diseased plants. Males and females both transmit the disease and with approximately equal efficiency. Adults from diseased bushes were able to transmit tthe disease, while bugs from healthy bushes less than 150 yards distant did not. There is no evidence that bugs from healthy bushes were rendered infective by feeding upon leaf of diseased plants. A larger number of infections occurred (except in one case) where the test feeding period was of six hours' duration or longer. An attempt to determine the most favourable test feeding site gave a small number of positive cases where the second youngest leaf was used. No evidence was obtained for the ability of a single bug to transmit the disease to a succession of plants. Experiments to show the effect of infection feeding time on transmissions, the persistence of virus in the vector, the existenc of a latent period in the latter, and the rate of spread of virus from the infection site, were unsuccessful.

An analysis of results obtained in six experiments where potted plants were held under insectary conditions is given in Table 11.

It is seen that the sudden die-back condition was acting independently of contact with *Oliarus* adults.

TABLE 11.—Analysis of Experiments with Potted Plants.

	No. of plants	Percentages of plants		
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Tests	739	2·7	4·2	8·8
Controls (no bugs)	269	2·2	0·4	8·9

THE INFLUENCE OF CAGING TECHNIQUES UPON ADULT INFECTIVE CAPACITY

In the work described above, the original caging techniques (Cumber 1953a) have been adapted to enable large numbers of plants to be handled. Although the bugs appeared to feed actively and lived for fairly long periods, the low number of positive infections showing up made it evident that either the populations differed from pervious years in their infective capacities or techniques were less successful. The following experiment was set up to gain further information.

Experiment 12.—Set up 25/11/52–3/12/52, and analysed 4/8/53.

Eighteen-month-old field-raised seedlings were used. Bugs from a severely affected plantation (area 6) were collected and randomized, except in the case of massed caging ("G" below) which was set up on the second day with bugs from the same area. Ten bugs per plant were used, and at the conclusion of the 8-day test feeding the plants were removed to a district 20 miles distant and planted out. Those set up in pots were allowed to remain there, the pots being embedded in the soil, but those not set up in this manner were planted directly in the soil.

DETAILS OF CAGING TECHNIQUES

A—Recently potted plants; wire gauze (12 meshes to inch) tubes, 8 in. by 2 in.; cotton-wool plugs top and bottom; access to all leaves between heights of 4 and 10 inches.

B—Recently potted plants; wire gauze tubes, 5 in. by 1 in.; cotton-wool plugs top and bottom; access to 3–4 youngest leaves at base of plant.

C—Plants standing with roots in water; cages as in A and commencing 3 in. above base of plant.

D—Plants standing with roots in water; cages as in B.

E—Plants standing with roots in water; bugs caged with entire test-tubes over third youngest leaf; tubes plugged with cotton-wool.

F—As in E, but sealed ends of tubes removed and gauze covers substituted.

G—Plants standing in small drum (1 ft. high, 6 in. diam.) with roots in water; mass caging of 10 plants with 100 bugs; conical gauze cover over drum.

Results are shown in Table 12.

TABLE 12.—Influence of Caging Techniques upon Numbers of Infections.

Caging technique	No. of plants	Plants			% Bugs	
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms	surviving	escaping
A	20	1	6	0	36	52
B	20	1	6	0	21	62
C	20	0	5	0	53	38
D	20	0	5	0	30	44
E	20	2	0	0	12	0
F	20	0	2	0	15	0
G	10	4	1	0	?	?
Controls potted: (no bugs)	20	2	0	0	—	—
Controls non-potted: (no bugs)	20	0	0	0	—	—

It is apparent that the caging techniques adopted influenced the numbers of infections. The low numbers of these obtained in experiments on adult transmission would appear to be due to techniques rather than to changes in the infective capacity of the field samples. The ideal cage will be one which is fairly small, is adequately ventilated, and prevents escape of bugs.

NYMPHAL TRANSMISSION

The majority of nymphal nourishment is taken during root feeding, but it is possible that there may be some feeding directly on the leaf bases and perhaps on the rhizome itself especially during the later instars. Nymphs are known to draw on the conducting elements of the roots, for if transverse sections are made where these pass through the nymphal galleries, numerous feeding puncture traces may be seen radiating outwards from the vascular bundles.

Experiments set up to test whether or not nymphal transmission occurs were designed to give additional informatiodn on disease-vector relationships should success in transmission be obtained. In preliminary

trials set up 14 September 1951, nymphs appeared to feed satisfactorily on leaves as well as on roots.

In the experiments which follow the seedlings used were raised under conditions where the possibility of infection with *Oliarus* was considered remote. Little mortality was observed amongst bugs during test feeding periods. In experiments 13, 14a, 14b, adequate screening was maintained throughout. The remainder of the experiments were kept in a glass-house until the summer of 1952–53, when it was considered that damage from mealy-bugs and spider mite would be reduced by placing plants out of doors. Restricted insectary space necessitated their being in the open and thus incurring some risk of field infection. Occasional adults of *Oliarus* were found despite separation by half a mile from the nearest plantation. Prior to their removal from the glass-house, some plants showed sudden die-back symptoms, but nothing comparable with the quickly developed and typical *Oliarus*-induced symptoms was seen.

Experiment 13.—Transmission through root feeding: set up 4–18/9/51, analysed 2/7/53.

Bugs were collected from diseased and healthy bushes, and allowed to feed on 18-month-old seedlings established in pots. The soil from the base of the plants was removed to expose the upper roots, and a layer of old dead *Phormium* leaf fragments placed over them. Nymphs were added, and muslin sleeves tied about the tops of the pots and around the bases of the plants prevented escape. After 14 days the bugs and leaf fragments were removed, the pots topped up and transferred to the insectary. Control plants were treated likewise apart from the application of bugs. Details and results are given in Table 13.

TABLE 13.—Transmission through Root Feeding.

Source of nymphs, bushes	Instar	No. of bugs per plant	No. of plants	Plants		
				died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
diseased	2 + 3	20	10	0	0	0
diseased	5	10	10	1	0	1
healthy	2 + 3	20	10	0	0	0
healthy	5	10	10	0	0	1
Controls (no bugs)	—	—	25	0	0	1

This provides no evidence for nymphal transmission through root feeding.

Experiment 14a.—Transmission in different instars: set up 6–10/9/51, analysed 2/7/53.

Bugs were collected from diseased and healthy bushes and caged on leaf of 6-month-old seedlings by means of test tubes with cotton-wool stoppers, the roots of plants being kept moist with cotton-wool. After a test feed of 7 days, seedlings were planted out in boxes and held in the insectary. Details and results are given in Table 14A.

TABLE 14A.—Transmission by Different Instars.

Source of nymphs, bushes	Instars	No. of bugs per plant	No. of plants	Test feed site	Plants		
					died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
diseased	2	1	10	blades	0	0	1
diseased	3	1	10	blades	0	1	1
diseased	2 + 3	4	8	blades	5	0	0
diseased	5	1	15	blades	1	1	4
diseased	5	4	8	blades	2	0	1
diseased	5	4	9	all plant except distal roots	1	0	2
healthy	2	1	10	blades	1	0	1
healthy	3	1	10	blades	0	0	3
healthy	5	1	15	blades	1	0	2
healthy	5	4	20	blades	1	0	0
healthy	5	4	5	all plant except distal roots	0	0	1
Controls (no bugs)	—	—	39	—	0	0	4

There is some evidence of transmission, but the number of infections is too low to draw definite conclusions. The proportion of sudden die-back symptoms, and of plants dying during experiments

are higher amongst those plants receiving nymphs from diseased bushes.

Experiment 14b.—Transmission in different instars: set up 5–8/10/51, analysed 8/7/53. This experiment includes fourth-instar nymphs, and tests the effect of a pre-test feed starvation period.

Bugs from diseased bushes were caged on the leaves of 6-month-old seedlings with test tubes and cotton-wool stoppers for a period of 6 days. The roots of plants were kept moist with damp cotton-wool. The seedlings were then transferred to boxes. Details and results are shown in Table 14B.

TABLE 14B.—Transmission in Different Instars.*

Nymphal instar	No. of bugs per plant	No. of plants	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
2	20 (20)	30 (20)	5 (4)	7 (3)	1 (0)
3	20 (20)	30 (20)	3 (2)	5 (3)	3 (1)
4	12 (20)	6 (20)	0 (1)	0 (3)	1 (1)
5	20 (20)	30 (20)	3 (4)	3 (1)	1 (0)
Controls (no bugs)	—	20 (20)	4 (2)	3 (1)	1 (0)

*In the case of bracketed figures a twenty-four hour starvation was given before test-feeding.

Some evidence for transmission is shown in the higher percentage of typical *Oliarus*-induced symptoms in test plants.

Experiment 15.—Ability to infect a succession of plants: commenced 24/9/51, analysed 15/7/53.

The experiment commenced with 40 fifth-instar nymphs from diseased bushes. They were caged singly by means of 4 in. by $\frac{1}{2}$ in. glass tubes on all roots and the lower halves of the blades of 6-month old seedlings. A little water in the bottom of the tubes kept roots moist and caused no apparent inconvenience to the bugs. Each bug was given a single-day test feed on 4 successive plants, then a 3-day test feed on 2 successive plants, then a 6-day feed on 1 plant, and thereafter 14-day feeds per plant. Twenty-two adults emerged, and on an average 9 plants were given to each bug. After test feeds plants were transferred to boxes.

No conclusions could be drawn from the final analysis. The percentages of plants affected are shown in Table 15.

TABLE 15.—Percentages of Affected Plants in Experiment 15.

	Total No. of plants	Plants		
		died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Tests	377	23	8	15
Controls	110	15	8	19

Experiment 16.—Persenstence of infective capacity: set up 19/9/51–10/10/51, analysed 9/7/53.

Bugs from diseased bushes were starved for varying periods then test fed on 6-month-old seedlings in test tubes. During the 5-day test feed they had access to the basal half of leaves and all roots. A little water kept roots moist. Plants were subsequently transferred to boxes. Details and results are shown in Table 16.

TABLE 16.—Persistence of Infective Capacity. *

Starvation period (days)	No. of plants	No. of nymphs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
0	20 (20)	4 (2)	6 (4)	0 (0)	4 (6)
1	20 (20)	4 (2)	7 (3)	0 (5)	2 (2)
2	20 (20)	4 (2)	4 (7)	3 (1)	2 (4)
4	20 (20)	4 (2)	9 (6)	0 (2)	2 (2)
8	20 (20)	4 (2)	4 (6)	2 (0)	3 (4)
16	20 (20)	4 (2)	5 (4)	4 (4)	7 (4)
Controls (no bugs)	120	—	26	7	21

*Unbracketed figures refer to experiment with second plus third instar nymphs; bracketed figures to fifth-instar nymphs.

Because of the number of *Oliarus*-infected plants in the controls it is considered that no evidence for persistence of infective capacity was obtained. Test plants considered together showed a higher percentage of typical *Oliarus*-induced yellow-leaf than did the controls,

Experiment 17.—Effect of infection feeding on transmission: set up 12–19/9/51, analysed 10/7/53.

Fifth-instar nymphs collected from healthy bushes were starved for 6 days in petri dishes containing moist filter paper. Infection feeds were then given (as in Fig. 2) for 48 hr. on leaves of diseased and healthy pot plants from the previous season's trials. Test feeds of 5 days were then given on 6-month old seedlings in test tubes with damp cotton-wool plugs. Plants were then transferred to boxes. Details and results are shown in Table 17.

TABLE 17.—Effect of Infection-feeding on Transmission.

Infection feed	No. of plants	No. of nymphs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Diseased plants	12	2	1	2	0
Healthy plants	15	2	0	6	1
Controls (no bugs)	20	—	2	1	1

There is no evidence for nymphs being rendered infective by feeding on leaf of diseased plants. It is noted that the greatest percentage of typical *Oliarus*-induced symptoms occurs in the plants receiving bugs given a healthy infection feed.

Experiment 18.—Effect of test feeding site on transmission: set up 18–21/9/51, analysed 14/7/53.

Bugs from diseased bushes were caged by means of test tubes on 6-month-old seedlings in the following ways: a. with access to all but the upper portions of leaves, b. on roots only, c. on leaves only. Bugs from healthy bushes were caged as in a., as were also the control plants receiving no bugs. After 3-day test feeds, plants were transferred to boxes. Details and results are shown in Table 18.

TABLE 18.—Effect of Test-feeding Site on Number of Transmissions.*

Infection feed	No. of plants	No. of nymphs per plant	Plants		
			died during experiment	with typical *Oliarus*-induced symptoms	with sudden die-back symptoms
Whole plant except upper leaf	15 (30)	5 (2)	9 (15)	1 (3)	1 (5)
Roots only	15 (30)	5 (2)	12 (11)	0 (4)	2 (5)
Leaves only	15 (30)	5 (2)	7 (16)	0 (2)	4 (4)
Controls—healthy feed	15 (30)	5 (2)	8 (14)	0 (6)	5 (6)
Controls—no bugs	15 (30)	—	4 (18)	1 (2)	5 (4)

*Unbracketed figures refer to tests with second- plus third-instar nymphs; bracketed figures to fifth-instar nymphs.

As in the preceding experiment, it is seen that the greatest number of plants showing typical *Oliarus*-induced symptoms occurred where

a healthy control feed was given. The numbers of affected plants differed little between the controls taken together, and the test plants.

SUMMARY OF RESULTS FROM NYMPHAL TRANSMISSION EXPERIMENTS

No significant information regarding the vector relationship in nymphal transmission has been obtained. Results have been complicated by the large numbers of plants affected with sudden die-back, and of dead plants. The number of plants and bugs involved in the combined experiments, does however, enable more reliable conclusions as to whether nymphs are or are not agents of transmission.

The more important findings have been noted following the description of each experiment. The anomalous result in Experiments 17, 18, where bugs from healthy bushes, given feeds on healthy plants, have produced a greater number of infections than bugs directly from diseased bushes, is of interest and has its parallel in Experiment 5 where adults were used.

Table 19 shows the percentage of infections in experiments involving the use of test plants receiving bugs from diseased bushes, and controls receiving no bugs. Dead plants, and those with sudden die-back symptoms, have been considered together. It is seen that in no experiment does the percentage of control plants showing typical *Oliarus*-induced symptoms exceed that in the tests, and in only one case in the controls does the percentage of dead plants plus plants with sudden die-back symptoms exceed that in the tests.

It seems probable, therefore, that the caging of nymphal stages on *Phormium* seedlings eventually results in an increase in the number of plants dying and being affected with symptoms of the yellow-leaf disease typical of those produced using adult bugs from infected bushes. Whether the bugs themselves transmit the pathogen or merely render plants more susceptible to one which is commonly present, cannot be determined until experimental conditions permit the maintenance of healthy control plants.

TABLE 19.—Percentage of Infections, Plants Receiving Nymphs from Diseased Bushes and Control Plants.*

No. of experiment	No. of plants	Total bugs used	Plants	
			with typical *Oliarus*-induced symptoms	with sudden die-back plus died during experiment
13	20 (25)	300	0 (0)	10 (4)
14a	60 (39)	135	3 (0)	30 (10)
14b	176 (40)	3,472	16 (10)	17 (17)
15	377 (110)	40	8 (8)	37 (27)
16	240 (120)	720	9 (6)	44 (39)
18	135 (45)	305	7 (7)	68 (69)

*Control plants are bracketed.

DISCUSSION

Confirmatory evidence for the virus hypothesis in the form of increasing infective vapacity by leaf feeding on diseased plants has not been obtained, and were it not for the evidence of Boyce et al. (1953)

from mechanical and graft transmissions, there would appear to be some grounds for suspecting that *Oliarus* might be carrying or increasing the susceptility to a fungal or bacterial disease.

If *Oliarus* transmits a virus, then the conditions of transmission would appear to be specialized and not adequately covered in the techniques adopted in the present work. There is no doubt that the length of life of the adult bugs handled in experimental work was reduced. Given optimum conditions it should be possible to keep them alive for almost a month. It the bug becomes infective only after a long latent perior, the results obtained might be explained.

The large number of plants that died, or were affected with the sudden die-back condition, has complicated assessment. At the time of analysis this condition was recognized as distinct from *Olairus*-induced yellow-leaf (Cumber 1953b). But it is not beyond the bounds of possibility that both sets of symptoms are due to the one pathogen attacking through different channels. For example, the typical *Oliarus*-induced yellow-leaf might be caused by a fungal or other pathogen attacking through the leaf bases, and the sudden die-back by the same pathogen gaining entrance to the rhizome via roots or cortex.

In experiments using adults, it has been shown that varying percentages of transmissions were obtained when different caging techniques were used. In future virus-vector relation experimental work special care should be given to adopting ideal caging techniques. Use should be made also of long test-feeding periods. If anomalous results are still obtained, the possibility of a part played by fungal attack should be examined further. There would be additional justification for this if the yellow-leaf disease were found to persist in the absence of *Oliarus*.

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CORRIGENDUM

Vol. 35 B, p. 421, for line 8, under "The New Zealand Ignimbrites," substitute: "evidence supports the hypothesis that the old arcuate structure of"