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Statistics Department

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increases in concentration at the onset of rain, and tests showed that water drops falling at their terminal velocity can disperse many spores. When the target was *Puccinia graminis* on wheat straws some of the spores were borne in splash droplets, but trapping on slides half coated with naphthol green B in gelatin and half with "Vaseline" showed that most were dry (see *Trans. Brit. mycol. Soc.* 44, 138–139, 1961). Further tests showed that spores are dispersed, with varying ease, from plants infected with: *Cladosporium herbarum, Alternaria* sp., *Ustilago tritici, Pseudoperonospora humuli, Phytophthora infestans,* conidia of *Venturia inaequalis, Erysiphe* sp., *Puccinia glumarum* and *P. obtegens.*

The number of spores released depended not only on the form and position in which they were presented but also on the size of the drop and the number which had fallen previously. No doubt many spores were released by the collisions shaking the plants and small glass beads were as effective as water in dispersing *P. graminis*. When *Lycopodium* spores were deposited evenly over the surface of a 5-inch-diameter steel block weighing $8\frac{1}{2}$ lb., beads suspended many fewer spores than water drops of either the same weight or diameter. None of these impacts shook the block, so it seems possible that dispersal was caused by rapid air movement ahead of the radially spreading drop during the millisecond after collision. (Hirst and Stedman.)

Soil activity of Phytophthora infestans

The tuber-slice technique for assessing *P. infestans* in soil (*Rep. Rothamst. exp. Sta.* for 1960, p. 124) was calibrated using black fen soil from Littleport, Cambs. The results differ by a factor of about 10 from those obtained with a Rothamsted soil. To infect 50% of the octants needed about 50 sporangia per slice, compared with 500 for Rothamsted soil, and 78% (or, after applying the multiple infection transformation, 150 infections/100 octants) were caused by 110 sporangia/slice, compared with about 1,000 with Rothamsted soil. Calibration is thus necessary with each soil tested.

Many antibiotics were found to be toxic to *P. infestans*, but pimaricin, polymixin and penicillin at concentrations of 100, 50 and 50 p.p.m. respectively have little effect on growth. Attempts to use these three antibiotics together, to check bacterial and fungal contamination when isolating the fungus from soil, failed because they did not control bacteria.

Soil samples were taken from around inoculated seed tubers of different varieties at intervals between 10 May and 4 August. Tuber-slice tests were positive from 18 May (about 3 weeks after planting) until 14 July, by when most tubers were rotting. Irrigation had little effect on infectivity. Ulster Ensign gave the most tuber-slice infections, followed by Duke of York, King Edward, Pentland Ace and Majestic, in order of decreasing numbers. Sporangiophores and sporangia were seen on tubers of Ulster Ensign and King Edward on several occasions. Spore production by inoculated tubers of Ulster Ensign was examined in the field and laboratory. Tubers were lifted at intervals from 6 to 21 days after inoculation in the field and incubated at 15° C. in a humid chamber for 24 hours. Sporangia were then washed off and their numbers

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largely illusory. From the acid-sprayed plots only 0.5% of the tubers were infected, whereas 10% of the others were. These samples point the need for further work on the best time to kill the haulms with acid; they show that, with such a susceptible variety as King Edward, even in a year when blight was generally regarded as unimportant, increased yields from copper sprays can be more than offset by tuber infections, which destroyed more than a ton per acre unless the haulms were killed by acid while still growing. (Hirst and Stedman.)

Apple scab

Spore trapping continued during the spring in one Bramley's Seedling orchard (Wisbech H, ascospore dose 20, see Hirst & Stedman, Ann. appl. Biol. 49, 290, 1961), but despite the exceptionally wet, late summer of 1960 ascospores were rare. Measurements of leaf survival and ascospore productivity were continued in several orchards.

Three investigations begun in previous years were extended. Catches of ascospores from heavily infected Bramley's Seedling leaves placed close to and level with the orifice of a Hirst spore trap $\frac{1}{2}$ m. above ground level represented up to 27,000/cu. m. of air. Because the source was locally so intense, it was possible to detect small releases during periods of dew deposition and to confirm the claim by Miller and Waggoner (Phytopathology, 48, 416-419, 1958) that some release occurred in fair weather, although in our observations it always followed nights with rain or dew. In these tests and a series where batches of leaves were repeatedly wetted in the wind tunnel, many more ascospores were liberated by day than by night. From other work it now seems unlikely that this results from single effects of light, temperature, atmospheric turbulence or the interval between wettings. There is evidence that the day-time prevalence, often noticed in orchards, results from the combined effects of repeated wetting and different responses of ascospore liberation and maturation at low night temperatures.

Previous measurements of the ascospore productivity of dead leaf samples from orchards suggested that sulphate of ammonia applied as a fertiliser before bud-burst might be decreasing the number of ascospores liberated. (Strong solutions of sodium nitrate or ammonium sulphate were found to have this effect by Keitt and others during their early work on eradicant fungicides.) In two tests in 1961, 3 or 6 cwt./acre of sulphate of ammonia given 3 weeks before bud-burst decreased the number of ascospores liberated 2 weeks later by more than 90%. No rain fell during these 2 weeks, but if the effect is confirmed in other weather, applications of sulphate of ammonia should be timed to make best use of their fungicidal and nutritive actions. (Hirst and Stedman.)

Raindrop collision with plant surfaces as a cause of dry spore liberation

Raindrops trap and deposit many fungus spores suspended in air, cause the discharge of some, by wetting the fructifications containing them, and disperse others in splash droplets when they collide with spore-bearing surfaces. Spore traps often show brief

Professor Marchal nil, Cappelle nil. The infection developed late, and the variety Viking yielded as much as Professor Marchal and more than Cappelle. (Slope.)

Effect of herbicides on take-all in wheat

Severe attacks of take-all on commercial farms are sometimes attributed to the use of herbicides of the hormone type, but as all the crop is usually sprayed, there is no direct evidence that the herbicide has affected disease incidence or severity. The apparent correlation between herbicide and disease need mean only that weedy fields are those with a history of cereal cropping, and are therefore liable to have take-all.

On Great Field I none of three herbicides affected the incidence of take-all in Cappelle wheat sown on infested land. During May and June numbers of infected crown roots were similar with all treatments, and in July the percentage straws severely affected was 37 in the unsprayed plots, 38 sprayed with 2:4-D, 30 with CMPP and 40 with MCPA/TBA. Spraying with herbicides had no significant effects on yields, which were small with the high incidence of take-all, or on height of straw or percentage of tail corn. The lack of effect in this experiment contrasts with an experiment on Highfield in 1957, when spraying Koga II wheat with CMPP doubled the percentage of severely infected straws; the increase then was from 14 to 26% on land where the experiment followed wheat and from 8 to 16% where it followed oats. (Salt.)

Control of potato blight

For the first time potato blight was recorded in a tuber in a growing crop before it was noted on the haulm of this or any other nearby crop. The event happened while sampling an experiment on the Rothamsted Farm on 8 August, when a young King Edward tuber was found infected on a plant otherwise free from symptoms. Dry weather delayed the "outbreak" stage (0.1% foliage de-

Dry weather delayed the "outbreak" stage (0.1%) foliage destroyed) until 15 September. As a result, the planned schedule of protective and haulm-killing sprays could not be completed on an experiment designed to determine the best date for "burning off". Instead, on 21 September, when blight had destroyed only 1% of the foliage, selected plots were sprayed with concentrated sulphuric acid to see whether yield was decreased by spraying with copper in a year almost free from blight. Some plots were not sprayed with acid, to measure the effects of later blight spread on plants with and without protective fungicides.

Although on 21 September the foliage was greener on the unsprayed plots than on those sprayed with copper, all those killed with acid at this time yielded similarly (13.57 and 13.86 tons/acre with two and three sprays with copper oxychloride and 14.12 without copper). Yields of plots not sprayed with copper were the same whether destroyed by acid on 21 September (14.12 tons/acre) or allowed to die on their own (13.95), but plots sprayed three times with copper yielded 14.98 tons/acre when allowed to grow to maturity, compared with 13.86 when killed with acid on 21 September. However, examining samples of lifted tubers showed that this apparent gain from copper sprays and loss from acid spray was

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Take-all and eyespot on Broadbalk

In July take-all was again less severe on Broadbalk than on the cereal-beans experiment, although early infection was similar on the two sites. In the 4th successive wheat crop on Broadbalk plot 7, 11% of plants were infected on 1 May and 26% on 17 July. In contrast, on the 5th wheat crop in the cereal-beans experiment 16% plants were infected on 14 April and 93% on 18 July. Routine sampling on Broadbalk in 1961 showed that on plots 2B, 3 and 7, the 1st, 2nd, 3rd, 4th and 10th consecutive wheat crops drilled on 19 January, after 1 year fallow, had respectively: 9, 49, 46, 46 and 31% straws with eyespot, of which 3, 24, 28 and 12% had severe lesions: 0, 9, 16, 19 and 9% straws had take-all on the roots. For the 5th consecutive year there was less eyespot in the continuous wheat (Section 1A) than in the 2nd, 3rd and 4th wheat crops after a 1-year fallow. (Cox.)

Brown Foot-rot (Fusarium spp.)

In 1959 and 1960 yields of spring wheat and responses to nitrogen were very small in some experiments with nitrogenous fertilisers at Woburn: the low yields were associated with high incidence of Brown Foot-rot. In July 1961 it was apparent that yields and responses to nitrogen would be small in an experiment testing rates and methods of application of nitrogen. This experiment was sampled on 11 August and the following incidence of Brown Foot-rot recorded: nil N 10%; 0.40 cwt. N/acre 39%; 0.77 cwt. N/acre 70%. The incidence of foot-rot was the same whether the nitrogen was broadcast as sulphate of ammonia or combine-drilled as part of a compound fertiliser. The mean grain yields were: nil N 9.4; 0.4 N 12.2; 0.77 N 11.1 cwt./acre. In each of the 3 years observations indicated that the poor growth and yield of wheat and the high incidence of foot-rot were associated with poor soil structure. (Slope.)

Cephalosporium stripe

The Cephalosporium isolated from wheat last year was confirmed as C. gramineum Nisik., Mats. & Yam. by Mr. J. J. Elphick of the Commonwealth Mycological Institute. In March Koga II wheat seedlings (three-leaf stage) were inoculated with this isolate by dipping their roots into a suspension of spores and mycelium before replanting; 92% of these inoculated plants developed typical symptoms of Cephalosporium stripe during April, and C. gramineum was easily re-isolated from them. Naturally infected plants were collected during May and June from the area of Broadbalk field where the disease was first found last year, but none could be found in other parts of the field. One infected plant was found in Deacons field. The disease has now been found in four fields at Rothamsted, but is too rare to be important. However, naturally infected and inoculated plants yield little or no grain, and should the disease become common, it could be extremely serious. (Slope.)

Yellow rust (Puccinia glumarum)

In a wheat variety trial on Deacons field the percentage of flag leaves infected by *P. glumarum* on 28 July was: Viking (S.80) 45,

Previous crops		Grain yield (cwt./acre) Third-year crop:			% straws with take-all spring wheat		
		1959	1960	1961	1959	1960	1961
W	W	32.0	28.7	32.7	12	26	20
sW	W	32.4	26.4	33.4	8	36	27
0	W	29.2	24.7	32.3	20	62	33
W	0	32.0	31.3	36.7	3	2	5

W = winter wheat; sW = spring wheat; B = spring barley; O = spring oats.

Take-all was the most prevalent disease, and weeds flourished only in crops severely stunted by disease. O. graminis var. avenae has not been recorded at Rothamsted, and oats therefore behave as a non-susceptible crop in this experiment. In the years when takeall was severe (1958, 1960) winter wheat after oats yielded 80%more grain than wheat after wheat, but only 12% more when takeall was not severe (1959). In the years 1959–61 take-all was less severe on spring wheat than on winter wheat; consequently, the effect of a previous oat crop on yield of spring wheat was much smaller. However, in 1958 a spring wheat crop in this experiment had 75% straws with take-all and yielded only 11.8 cwt. grain/acre.

The fourth-year crop (Cappelle wheat, sown 23 January on Series II) had 58, 76, 71, 66, 72 and 7% straws with take-all in July after the crop sequences W W sW, B W B, sW W sW, O W sW, W O sW and W O Beans respectively, and yielded 29.3, 22.0, 27.6, 23.7, 25.2 and 43.4 cwt. grain/acre. (Slope.)

Effect of chlorinated hydrocarbons on take-all

In pot experiments mixing aldrin (A), dieldrin (D), chlordane (C) and heptachlor (H) with soil before planting decreased infection of wheat inoculated by burying agar cultures of O. graminis 21 inches below the soil surface. Cappelle wheat, sown 31 January, had 40.4% crown roots infected in untreated controls on 28 March; adding A, C, H, at 0.005 g. active ingredient/pot (each pot contained 2.3 kg. soil) brought down the percentage roots infected to 36.3, 17.8, 29.3 respectively, and at 0.05 g./pot brought them to 11.4, 0.0, 1.7. An experiment sown with Koga II wheat on 28 April gave similar results. On 4 July 91.6% of the crown roots of untreated control plants were infected, but only 13.8, 50.1 and 2.7% of roots of plants grown in soil treated with 0.05 g. active ingredient of A, D, H respectively. Although the chlorinated hydrocarbons were used at high rates in these experiments (0.05 g./pot is equivalent to 18 lb./acre on an area basis), the amount of inoculum was much greater than would occur naturally, and the results seemed to justify field experiments. On 3 October Cappelle wheat was sown with 100 or 300 lb./acre of a 2% granular formulation of heptachlor. On untreated control plots on 2 November 23.6% plants had take-all; this was brought down to 19.0, 14.8 and 13.2% by heptachlor broadcast, combine-drilled or side-placed respectively; the percentage infection was similar for both rates of application of heptachlor. This decrease in primary infection is unlikely to be important unless the heptachlor also decreases later development of the disease. (Slope, with Last and Bardner, Insecticides Department.)

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The mean number of wheat straws per acre (1942-60) showed an annual variation between 1,106 and 2,091 in thousands per acre. Thirty-eight % of the annual variation in straw yield was accounted for by variation in the number of straws alone, the inclusion of eyespot having no additional effect. Only 18% of the variation in grain yield was accounted for by the number of straws alone, but when the depressing effect of severe eyespot was included together they accounted for 48% of the variation. (Glynne.)

Uncommon wheat diseases

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Gibellina cerealis. This rare and interesting pathogen on wheat is recorded in Britain only on Hoos field alternate wheat and fallow experiment. It was more common in 1961 than since it was first recorded in 1935, though it is unlikely to have materially affected yield. As in several other years, it was more common after 3 years than after 1 year under fallow.

Melanospora damnosa, in association with Fusarium culmorum, was found on oats in Sussex, on wheat, barley and oats at Rothamsted, on wheat near Hitchin and on barley in Hants. It had previously been reported in Sardinia and Tunisia, but this appears to be the first record on cereals in Britain. (Glynne and Dr. F. Joan Moore, Ministry of Agriculture, Fisheries and Food.)

Effect of date of sowing of winter wheat in relation to previous crops

Unusually heavy rain prevented sowing during October, November and December in the final year of the series of experiments on date of sowing of winter wheat. After two successive non-cereal crops (beans-potatoes) the mean grain yields of Cappelle wheat sown on 29 September (D1), 19 January (D2) and 15 February (D3) were 41.2, 48.5, 45.7 cwt./acre. Eyespot and take-all were negligible, and lodging was severe only on D1, which, on 22 July, was 13% lodged at N1 (0.6 cwt. nitrogen/acre) and 75% lodged at N2 (1.1 cwt. nitrogen/acre). After a wheat crop the mean yields were 28.5, 34.8, 37.7 cwt./acre for D1, D2, D3, respectively. Take-all (*Ophiobolus graminis*) and eyespot (*Cercosporella herpotrichoides*) were both decreased by late sowing; in July D1, D2, D3 had 65, 28, 18% straws with take-all and 18, 10, 5% with severe eyespot. There was little lodging. The responses to additional nitrogen (N2-N1) differed greatly on the two experiments; the responses on D1, D2, D3 were -3.4, +4.4, +0.1 cwt. grain/acre after potatoes and +12.2, +9.8, +5.0 cwt./acre after wheat.

Cereal-beans rotation

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The summarised results of the wheat yields in the second and third years of the rotations on all three series of this experiment are:

Previous crops	Grain	yield (cwt Secon		% straws with take-all winter wheat		
	1958	1959	1960	1958	1959	1960
W	17.4	37.4	25.2	89	11	84
В	19.3	31.9	30.2	82	41	82
sW	17.3	37.5	21.2	89	29	85
0	29.2 *	41.9	47.5	8	2	10
			•			

* Adjusted yield, severe bird damage.

and, in the north, only pea leaf-roll virus (PLRV) was common. There was a consistent difference between varieties in the incidence of PLRV but not of pea enation mosaic or pea mosaic viruses. At all sites the incidence was greater in the tick bean Herz Freya than in others and was often least in Minor; in horse beans incidence was greatest in Granton and often least in Strubes. At Rothamsted the primary infestation of all four varieties named above and three others by *Aphis fabae* and *Acyrthosiphon pisum* was similar, and was not correlated with the incidence of PLRV. Glasshouse experiments, in which young plants of these varieties were similarly infested with *A. pisum* taken from PLRV-infected plants, showed that Herz Freya was more susceptible than Minor, and Granton more than Strubes. (Gibbs, L. R. Taylor, Entomology Department, and Mr. D. T. A. Aldrich (N.I.A.B.).)

FUNGI AND FUNGUS DISEASES

Surveys of cereal foot and root rots, 1937-60

Wheat grown in the six-course rotation experiment (potatoes, rye, sugar beet, barley, clover, wheat) at Rothamsted had an average of 34% straws with eyespot (19% with severe lesions) in the period 1937–60. The proportion of straws infected varied in different years from 2 to 99%, those with severe lesions from 1 to 81%. This variation depended both on weather and on the amount of fungus surviving from preceding cereal crops. The steady rise in severe infections in the living wheat from a mean of 7% in the first 6-year period to 31% in the fourth is attributed to a gradual increase in infected crop residues during the experiment. The small incidence at the beginning probably reflects the fact that, on different blocks, the site had been free from wheat for 6–11 years before the experiment began.

The fungus (*Cercosporella herpotrichoides*) is helped to survive the 5-year interval between wheat crops in the six-course experiment by the cereal crops grown in alternate years. Barley, about as susceptible as wheat, is sown in spring, which allows less time for the fungus to develop; rye, less susceptible, but sown in autumn, develops about as much eyespot as barley. In the 9 years in which barley and rye were surveyed (1952–60) each had an average of 8%straws infected, but only 2% had severe lesions, as compared with 45% straws infected, 27% severely, in wheat during the same period. Self-sown barley in the following clover crop in some years also helped the fungus to survive.

Wheat lodged on some plots in 10 of the 30 years of the experiment (1931-60). It was severe only on plots which carried at least 51 cwt. straw/acre or when at least a third of the straws had severe eyespot lesions; when 44-81% were severely infected, some plots lodged with as little as 38-40 cwt. straw/acre.

The lowest yield of wheat grain was obtained in 1958, when straw yield was near the average (of 44.9 cwt./acre), but 81% of the straws had severe eyespot lesions, and the whole crop was lodged. Mean grain yield (30 years) was 28.5 cwt./acre, and the highest yield, 41.1 cwt./acre, was obtained in 1944, when eyespot was slight and there was no lodging or other recognised inhibiting factor.

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the discovery that inbreeding develops similar abnormalities in insects that are free from the virus. Thus when four females were brought in from the open, the eggs of three were all normal, and only a few eggs laid by the fourth failed to hatch. However, brother-to-sister matings among their progeny produced females whose eggs were 31% abnormal and failed to hatch; females produced by outbreeding laid only 4% abnormal eggs. Further inbreeding caused 48% abnormality in the F2 generation, compared with only 3% in outbred F2 females. Of the F2 inbred females, 6% were sterile or produced all abnormal eggs, and about 30% had fewer than 1 in 5 abnormal eggs. (Kisimoto.)

Poa annua plants in Great Field were found infected with wheat striate mosaic. Plants sent by Miss K. Ikaheimo from Finland also contained a virus apparently identical with ours. Plants brought by Dr. J. T. Slykhuis from Egypt, and thought by him to be infected with a virus like wheat striate mosaic that might have Sogatella nigerenis as a vector, yielded no virus when fed on by either S. nigerenis or D. pellucida. Neither S. nigerenis nor Euscelis plebeja transmitted wheat striate mosaic. (M. A. Watson.)

Adult *D. pellucida* became infective when juice from plants with striate mosaic was injected into their thorax at 5° C. There was an incubation period of from 10 to 23 days before the injected leaf-hoppers transmitted and about one-third of them survived long enough to transmit. Infective hopper extracts could be obtained at dilutions equivalent to one adult hopper (weight 3 mg.) per millilitre. Plant extracts diluted 1:2 infected hoppers, but higher dilutions did not. (Serjeant.)

Other grass and cereal viruses

Of twenty samples of cereals from different parts of England, Ireland and Scotland tested for viruses, eighteen, including the Irish samples, were infected. Sixteen had viruses of the barley yellow dwarf type transmitted by *Rhopalosiphum padi*, *Sitobium avenae* and *S. fragariae*, and two of the type transmitted by the *Sitobium* spp. and *Metopolophium dirhodum* only (*Sitobium* viruses).

Of seven samples of grasses tested, two contained cocksfoot streak, and one striate mosaic virus.

Barley yellow dwarf was recovered by *Ceruraphis eriophori* from sedges, *Carex trichocarpa* or *C. laxiflora*, sent by Dr. Eastop of the Natural History Museum. Both are new records for the virus.

The Sitobium-transmitted yellow dwarf viruses were recovered by Sitobium fragariae and less often by Metopolophium dirhodum when fed through membranes on sap from infected plants. R. padi fed in this way did not transmit these or any viruses; nor did the Sitobium spp. transmit the padi-transmitted viruses, although they do from plants. (M. A. Watson and Serjeant.)

Virus diseases of beans (Vicia faba)

The incidence of virus diseases was recorded in field beans at four of the spring-bean variety trials of the National Institute of Agricultural Botany, Cambridge, and also at Rothamsted, where seven varieties were grown.

Beans were infected later in northern than in southern Britain,

sown one to invasion by winged aphids. The spray checked the population of wingless aphids breeding in the crowns of the carrots, but had less effect on incoming winged ones, which, arriving a week after spraying, could feed long enough on the carrots to deposit young.

Transmission tests in the glasshouse show that motley dwarf is caused by the simultaneous infection of carrots by two viruses, redleaf and mottle. Red-leaf virus is transmitted by *C. aegopodiae*, but not by manual inoculation with sap from diseased plants; it has not been transmitted to any plant outside the *Umbelliferae*. Mottle virus is transmitted by manual inoculation of sap, has hosts outside the *Umbelliferae* and causes characteristic symptoms in *Nicotiana clevelandii* and *Chenopodium* spp. It is transmitted by aphids only from plants also infected with red-leaf virus, but from these can be transmitted by aphids to plants insusceptible to redleaf. Both viruses seem to be retained equally long by aphids, and both were still transmitted by aphids 8 days after they had left infected plants.

Carrot could not be infected by sap inoculation with mottle virus, but another umbelliferous plant, coriander, was. Transmission from carrot and other hosts of mottle virus were achieved by inoculation with sap, and with leaf extracts made with phenol-water mixtures. The infectivity of these extracts was probably conferred by virus nucleic acid, for it was destroyed by pancreatic ribonuclease which does not inactivate the virus in sap. Sap from plants infected with mottle virus contains specific spherical particles, diameter about 30 m μ .

About a quarter of the carrot seedlings raised from seed set by infected plants have reddened cotyledons or first true leaves. These seem to contain red-leaf but not mottle virus. Symptoms in seedlings that inherit the virus through the seed are mild and ephemeral, but virus transmitted from them by aphids to other seedlings often caused more severe and more lasting symptoms.

A virus obtained by Dr. R. Cammack from a mottled parsnip by sap inoculation caused symptoms in *N. clevelandii* resembling those caused by carrot mottle virus. However, it differed by being transmitted by sap inoculation to carrot and celery. This virus was not transmitted by *C. aegopodiae*, but in tests direct from mottled field parsnips the aphid transmitted virus to carrot and caused symptoms similar to, but not identical with, motley dwarf. Like carrots with motley dwarf, these parsnips probably contained two viruses, for the virus transmitted from them by inoculation of sap was not afterwards transmitted by aphids. (M. A. Watson and Serjeant.)

Wheat striate mosaic

Another vector was identified for wheat striate mosaic in $Delphacodes \ dubia$; after feeding for 8 days on infected plants, more than 70% of the individuals tested infected healthy plants on which they fed. (Kisimoto.)

We previously reported an abnormality in the eggs of *Delpha*codes pellucida infected with wheat striate mosaic virus. The role of the virus in this abnormality, however, becomes doubtful from