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PRELIMINARY EXPERIMENTS WITH APHIDS AS VECTORS OF YELLOW DWARF[1]

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Yellow dwarf is a comparatively new communicable disease of the cultivated onion and the literature pertaining to it is rather meager. Recently the writers (1) demonstrated the ability of certain species of plant lice to serve as agents for conveying this causal agent from diseased to healthy plants. In further work many significant facts concerning the transmission, development and behavior of the disease under greenhouse conditions have been determined.

Since the natural spread of yellow dwarf apparently depends solely upon its insect vectors, the rôle played by aphids in epidemics needs further perscrutation. Although many attempts to use the bulb mite (*Rhizoglyphus hyacinthi* Boisduval), the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood), the red spider (*Tetranychus telarius* Linn.), the onion thrips (*Thrips tabaci* Lind.), springtails (Collembola), leaf bugs (Miridae), leaf hoppers (Cicadellidae), onion maggots (Anthomyiidae and Ortalidae), and numerous other insects as vectors of the disease have been made, the experiments have given negative results, except in a very few cases with the six-spotted leaf-hopper (*Cicadula sexnotata* Fall) and in one instance with a mealy bug (*Phenacoccus* sp.) found on greenhouse plants. On the other hand, many careful experiments in the greenhouse with the bean-aphid (*Aphis rumicis* Linn.)[2], apple grain aphid (*Rhopalosiphum prunifoliae* Fitch), green peach aphid (*Myzus persicae* Sulzer), melon aphid (*Aphis gossypii* Glover), potato aphid (*Macrosiphum gei* Koch) and the corn leaf aphid (*Aphis maidis* Fitch) have positively shown that these species are conveyors of the yellow dwarf disease.

The present paper deals only with those experiments in which insects showed repeated ability to serve as vectors of yellow dwarf. In a subsequent article the experiments with insects which showed inability to serve as vectors of the malady will be discussed. Contrary to a statement in the recent literature the vectors of yellow dwarf are not confined to a local area in the state, but are widely disseminated in the United States and Canada. Some occur even on other continents. In the greenhouse studies the first transmissions of yellow dwarf were secured in December, 1930, by the corn leaf and the apple grain aphids.

A cursory examination of the publications relating to virus diseases of plants is sufficient to show the importance of members of the family Aphididae as active agents in the transmission of these diseases. Because of the unstable and at times almost insouciant feeding habits of the volant migrants, certain species of aphids are efficient carriers of the viruses of

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[1]Journal Paper No. B53 of the Iowa Agricultural Experiment Station, Ames, Iowa.
[2]Aphididae determined by Dr. F. C. Hottes.

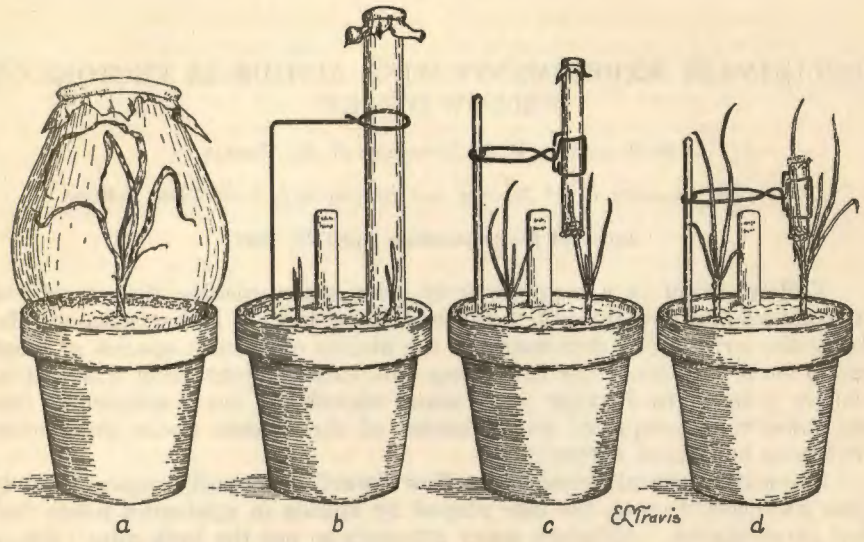

Fig. 1. Types of cages used for confining aphids on growing onions: *a*, aphids feeding upon diseased plant; *b*, *c* and *d*, cages adapted for confining viruliferous aphids upon entire or part of plants (control plants not caged).

many plant maladies. The behavior and migration of these insects are governed largely by their host specificities, periodic or seasonal host-restrictions, numerical abundance, high capacity for reproduction, and the availability of food plants. Some species are monophagous, whereas other forms are oligophagous or polyphagous and feed with various degrees of success upon many different kinds of plants during the growing season. Strangely enough, the secondary or summer hosts of polyphagous aphids serve only for parthenogenetic reproduction, for in the fall at the approach of cold weather, these species, like tourists returning from vacations, go back to the primary hosts. At this time or just before, the sexuparae appear and soon afterwards the sexuales. The oviparous females on the primary host then lay the eggs which serve to carry the species through the inclement season. Under field conditions in Iowa, most aphids spend the winter in the egg stage on branches of the primary host, which in the majority of cases is either a tree or shrub. In the experiments herein reported no work was done with the fundatrices, spuriae apterae, sexuales, or spring and fall migrants, the greenhouse studies being confined entirely to the agamic viviparous forms found on the summer host.

With favorable food plants and optimum conditions of temperature and relative humidity during the spring and summer, plant lice multiply very rapidly and often thickly populate and overrun their hosts. This overcrowding, together with the age and varying degree of succulency of food plants, tends to accelerate migration. In all species, however, there is in the winged individuals an inherent urge to wander so that under field conditions migration, which can neither be started, stopped, nor in any way greatly modified, occurs almost incessantly. Many graminivorous, herbivorous, and polyphagous species wander about in the spring, summer

and fall in search of new and less densely populated host plants, and thus tend to fill up any habitable gaps occurring in the environment.

In order to determine the distribution of the causal agent of yellow dwarf in the growing onion, aphids were confined on very limited sections (roots, bulbs, stems and various parts of young and old leaves such as the tip, central portion and base) of diseased sets and mother bulbs for a period of ten to twenty hours, after which they were transferred to healthy plants. In due time these showed unmistakable symptoms of the disease, thus proving that the infective principle resides in all these vegetative parts. Even wilting leaves, detached from diseased plants, served effectively as sources of inoculum. Parallel experiments, on the other hand, proved that healthy plants may be infected through the feeding of viruliferous aphids on the roots, bulb or stem, as well as on various parts of young and older leaves. Under certain field and greenhouse conditions the symptoms of yellow dwarf may be completely masked and not visible until after the bulbs have undergone a rest period and subsequently have been regrown. In numerous tests it was found that masked plants, both young and old, are capable of infecting plant lice, which, when so contaminated, can convey the disease to healthy onions. In this connection it should be noted that those leaves on diseased plants which show no external evidence of the disease are also capable of serving as sources of inoculum for plant lice. Viruliferous seedlings, sets, mother bulbs and commercial onions in which the symptoms are masked thus may serve not only as a means of carrying the disease from season to season, but also as a source of infection for in-

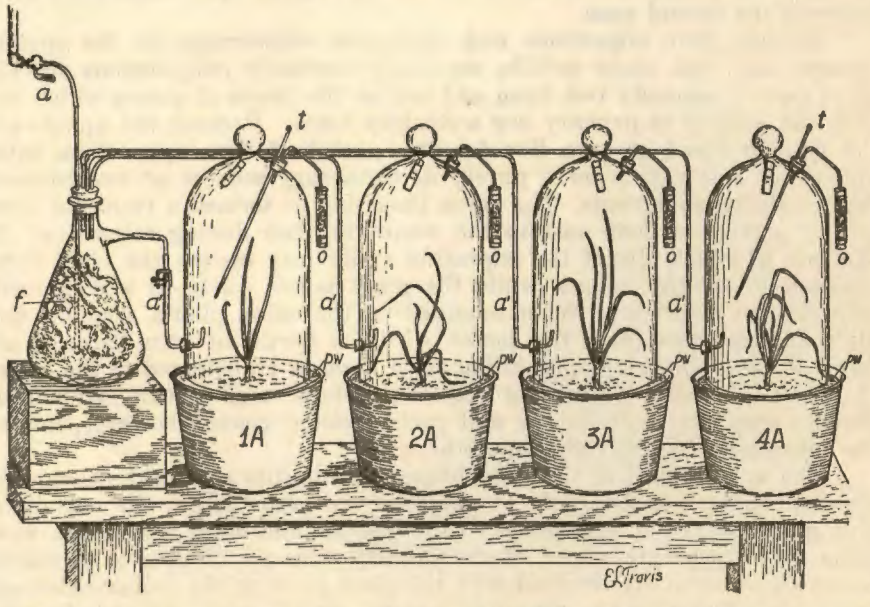

Fig. 2. Insect-proof cages: 1A and 3A, control plants; 2A and 4A, plants exposed to feeding of virus-bearing aphids; *a*, compressed air-line; *f*, cellulose cotton filter for removing oil from compressed air; *a'*, line for delivering air to cages; *o*, outlet of cages, showing cylinder containing glass-wool to keep out minute insects; *t*, thermometer.

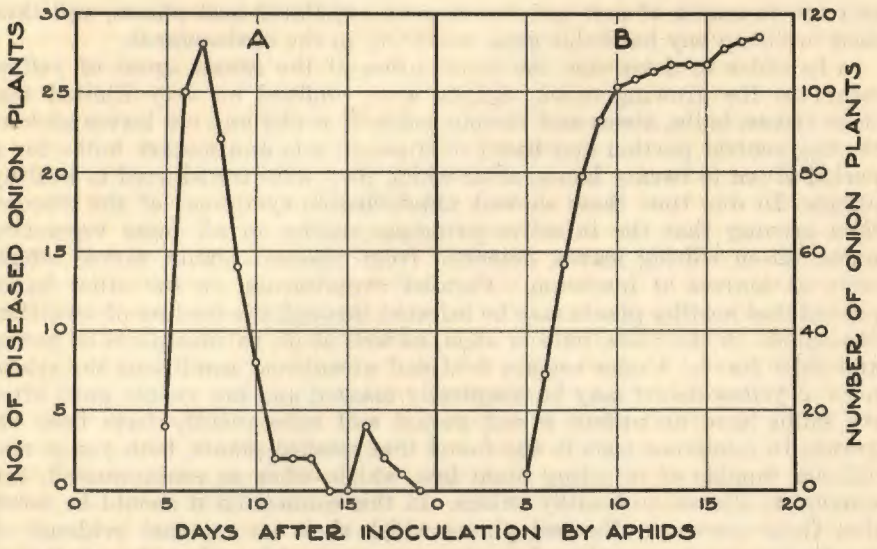

Fig. 3. Inoculation experiments in 120 set onions: A, showing daily initial expressions of yellow dwarf in 114 plants; B, total daily expressions of the disease in 114 out of 120 plants (6 plants failed to show symptom).

sect vectors during the first season as well as after the bulbs have been regrown the second year.

During their migrations and fortuitous wanderings in the spring, summer and fall, many aphids, especially markedly polyphagous species, by chance or necessity rest upon and imbibe the juices of plants which can function neither as primary nor secondary hosts. Because the aphids are not able to breed or even live for any period of time upon them these ephemeral host plants serve purely as temporary sources of nourishment for the shifting migrants. The onion thus may be termed a transient host, merely serving to help sustain the wandering lice during migration. In the case of yellow dwarf the migrating aphid may convey the virus from diseased to healthy onions, while the plant serves solely as a temporary host for the transients. When confined on the onion plants, nymphs and alate and apterous plant lice perish within a period of from three to six days; in fact, young nymphs, either born upon or transferred to the leaves of onion, die before attaining the adult stage, and mature viviparous females soon stop reproducing and perish. Some species, however, imbibe the juices much more freely than others.

The aphids used in the greenhouse experiments consisted of summer generations composed largely of apterous viviparae. Since the cultivated host plants were not susceptible to the disease, and since the aphids were colonized in separate rooms, there was little danger of aphids or onions becoming accidentally infected with the virus prior to the transmission experiments. From time to time non-infected aphids were caged on healthy onions as controls. Other plants not exposed to attacks of aphids were also used as controls. The total number of these control plants amounted to nearly twice that of the experimental ones, but in no instance did they

show any symptoms of yellow dwarf. In all of the experiments efforts were made to use young and vigorous aphids from small colonies feeding upon healthy hosts. At regular intervals new colonies were started so as to maintain a continuous supply of the insects. Yellow bottle-neck onion sets of a uniform size, grown at Clear Lake, Iowa, and free from yellow dwarf virus, were used in the experiments. In order to have a succession of young onions for experimentation some sets were planted every two or three days. After onions have been grown in a greenhouse for several weeks thrips become extremely abundant and make it imperative that a definite spray program be followed. The plants, therefore, were sprayed regularly at intervals of three days with nicotine sulphate or pyrethrum-soap solution to reduce the population of onion thrips. The experimental plants were caged only during the inoculating period. On the fourth day the cages were removed and both plant and cages were carefully sprayed to make certain that no plant lice escaped in the experimental room.

After healthy plants have been exposed to viruliferous aphids, the first visible expressions of yellow dwarf generally appear within a period of from five to twelve days. In a series of carefully planned experiments aphids were found to be very efficient conveyors of the virus of yellow dwarf. The results of some of these experiments are given in figures 3 and 4. Viruliferous aphids (Fig. 3) were confined on 120 plants and 114, or 95 per cent of these set onions, developed typical symptoms of the dis-

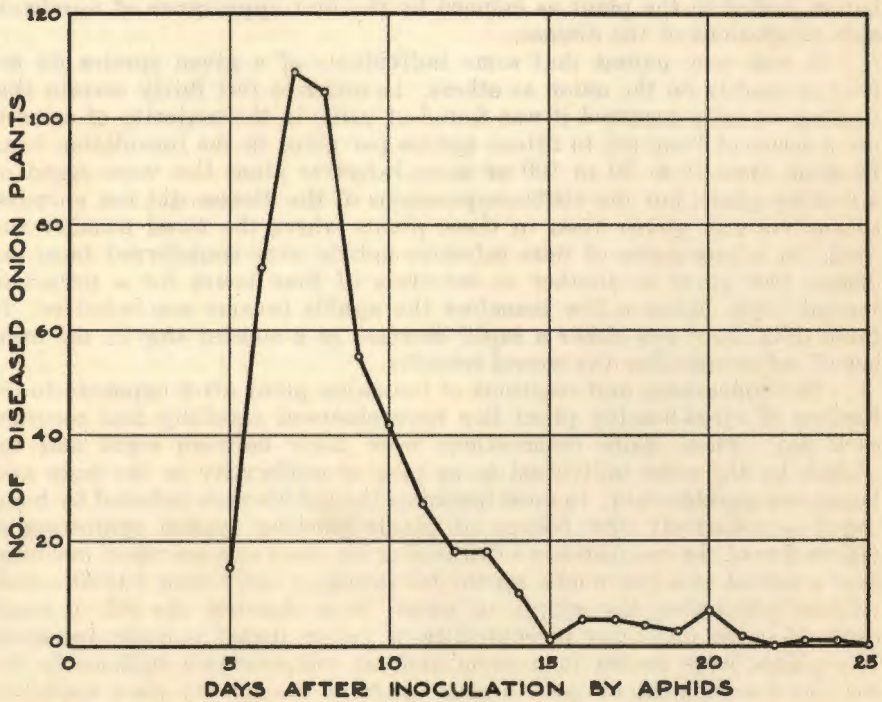

Fig. 4. Showing daily symptom expressions of yellow dwarf disease in 500 set onions inoculated by *Aphis rumicis*.

ease. The remaining six bulbs have not as yet been tested to see whether the disease was in masked form or whether the aphids failed to inoculate these plants. The length of time necessary for the appearance of the diagnostic characteristics of the disease in 500 transmissions to set onions by *Aphis rumicis* (Linn.) is depicted in the accompanying chart (Fig. 4). In this connection it is interesting to note that in slightly over 42 per cent (214) of the sets the first visible symptom expressions of the disease appeared on the seventh and eighth days after exposure to virus-bearing aphids. As was evidenced by a few stragglers, a small percentage of the plants had an arrested or prolonged development of the infection resulting in a temporary masking or restriction of the external manifestations of the disease.

In many tests using one, two, three, four and five infective aphids, respectively, it was found that one individual was capable of inoculating a plant, but the percentage of transmission under such conditions was much less than when a higher number was used. Aphids caged for thirty minutes on diseased plants and then transferred individually to healthy onions for a similar period of time successfully inoculated them, thus in a way demonstrating the extreme infectiousness of the disease, as well as proving conclusively that wandering plant lice may become carriers or, if already infective, inoculate plants by single feedings. In these preliminary tests using a few or an individual aphid as compared with those using a series of ten to fifteen there seemed to be no appreciable difference in the incubation period in the plant as evinced by the first appearance of unmistakable expressions of the disease.

It was very patent that some individuals of a given species do not feed as readily on the onion as others. In order to feel fairly certain that feeding actually occurred it was found of value in the majority of cases to use a series of from ten to fifteen aphids per plant in the inoculation tests. In some cases from 50 to 100 or more infective plant lice were caged on a healthy plant, but the visible expressions of the disease did not manifest themselves any sooner than in those plants where the usual number was used. In a long series of tests infective aphids were transferred from one disease-free plant to another at intervals of four hours for a period of several days. After a few transfers the aphids became non-infective. In these tests there was either a rapid decrease or a sudden stop in the number of infections after the second transfer.

The appearance and reactions of the onion plant after exposure to the feeding of virus-bearing plant lice were observed carefully and recorded each day. These daily observations were made between eight and ten o'clock by the same individual so as to give uniformity in the tests and, hence, comparable data. In most instances the aphids were infected by being caged on relatively new foliage of plants showing typical symptoms of yellow dwarf. In one instance a diseased onion, used as a source of inoculum over a period of a few weeks, served for infecting more than 10,000 aphids without exhausting the supply of virus; from this test the rôle a single diseased onion may play in epidemics of yellow dwarf is quite apparent. The plants were grown in a room kept at temperatures sufficiently low for the development of good onions, whereas the insects were reared in other rooms at higher temperatures favorable for their rapid multiplication. Everything considered, the months of February, March and April

were most suitable for these studies under greenhouse conditions. The greenhouse was lighted artificially (250 watt bulbs) on cloudy days and at night until eleven o'clock during the months of February and March.

The original culture of the bean aphid (*Aphis rumicis* Linn.) used in the long series of experiments was secured from Dr. F. L. Campbell, Bureau of Entomology, Washington, D. C. This race feeds on nasturtium and has been reared under greenhouse conditions for many years for toxicological and insecticidal tests by Richardson, Campbell, Shepard and others.

It is a well established fact that the virus of yellow dwarf overwinters in diseased sets, mother bulbs and commercial onions. Such bulbs, together with diseased scullions and culls thrown in refuse piles and dump heaps or left in the field, may serve as sources of inoculum for the vectors the following season. Because of the early development and appearance of typical symptoms in diseased bulbs regrown the subsequent spring, the relation of such bulbs to the spread of yellow dwarf is quite evident. Since no perennial wild host has as yet been found in the fields of Iowa, the diseased onion bulb appears to be the true reservoir of the overwintering virus.

Aphids are capable of conveying the infection to healthy onions almost immediately after having fed upon a diseased plant. Conversely, on the third or fourth day after being bitten by infective plant lice, the onion becomes a source of infection for aphids. After this short incubation of three to four days, diseased plants remain a constant source of inoculum as long as the aphids are able to extract the juices from them. Such diseased sets or bulbs, when regrown the following spring or left in the field after harvest to sprout and grow as volunteers, again become sources of infection for the vectors. On the other hand, aphids soon lose the virus unless constantly reinfected from diseased plants; after being caged on wild or cultivated hosts, or after a few transfers from healthy to healthy onions, they also become non-infective.

Viruliferous aphids were caged on set onions soon after the tips of the new leaves appeared. In many instances the first expressions of the disease showed on such sets while the bulbs were still in the three-leaf stage. Mature or nearly mature plants and slow-growing and unhealthy ones do not seem to be so easily infected as healthy and rapidly-growing young onions. More masking also occurs in the older plants than in newly started sets, mother bulbs and young seedlings. The degree of injury depends considerably upon the stage of growth and size of the bulb when the vectors inoculate its foliage. If infected soon after the new foliage appears, sets frequently fail to make any appreciable growth. The principal losses are due to the failure of diseased plants to manufacture and store sufficient food for the development of commercial onions. Since the actual sources of yellow dwarf infection at the beginning of each season are onion bulbs infected the previous year, and since the spread of the virus from diseased to healthy plants depends solely upon insect vectors, the control of the disease is largely a matter of preventing infection of the new crop from the overwintering virus and its subsequent wholesale dissemination by its insect vectors.

Spraying to prevent the spread of yellow dwarf by insect vectors is expensive and has extremely limited possibilities. Complete destruction of all crop remnants along with the use of disease-free sets produced in

non-infected areas constitutes the most practical remedial measure. Since the disease is not seed borne and since aphids are delicate flyers and also soon become non-viruliferous when removed from diseased plants, it is possible to produce disease-free sets within a few miles of infected fields. In the experiments for the control of onion thrips and maggots, many different combinations of spray materials have been used. Indexing of the sets produced in these experimental plots has disclosed that certain sprays had a noticeable effect in lessening the percentage of yellow dwarf infection. The foremost of these sprays was a combination of miscible oil (1-1.5 per cent) in bordeaux mixture (4-4-50) with pyrethrum soap (1-1000) or nicotine-sulfate (1-1000). Three to five applications of the sprays were made at weekly intervals. Those sprays which might have served to kill, or repel the aphids for several days, seemed to give better results than those containing more quickly-disappearing contact ingredients. In some of the check plots the infection with yellow dwarf was as great as 60 per cent, whereas in the plots sprayed with the above mentioned material infection was reduced to less than two per cent.

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PLATE I

Onion plants showing (a) typical feeding injury by the onion thrips and (b) yellow dwarf disease.

PLATE I

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