T. Pl.ziekten 64 (1958): 383–391

BENEFICIAL EFFECT OF VIRUS-DISEASED PLANTS ON NON-VECTOR INSECTS[1]

Met een samenvatting: Over het gunstige effect van viruszieke planten op insekten, die het virus niet kunnen overbrengen

BY

K. MARAMOROSCH
The Rockefeller Institute, New York, New York[2]

INTRODUCTION AND LITERATURE

Virus diseases of plants have been studied primarily because of their deleterious effects on plants. However, the history of variegated tulips infected with viruses (VAN SLOGTEREN, 1941; THUNG, 1949; DUBOS, 1958) presents a classical illustration of manifestations which have proved desirable at least from a commercial point of view. Several other cases of desirable virus infections are known to floriculturists, who have achieved beautiful effects of color breaking in different plants. Other desirable consequences of virus infections, manifested by attractive chlorotic or mottled patterns in leaves, can be seen in infectious chlorosis of *Abutilon* (ORLANDO & SILBERSCHMIDT, 1946; BAUR, 1904) and in the vein clearing of honeysuckle, *Lonicera japonica* (KUNKEL, 1958). The above cases illustrate "usefulness" of certain virus infections in respect to *Homo sapiens*. Do other species of animals also profit from virus infections of plants? There are several accounts in the literature, indicating increased longevity of insect vectors on virus-infected plants. SEVERIN (1946) observed an increased number of beet leafhoppers *Circulifer tenellus* BAKER on sugar beets infected with curly top virus. SEVERIN's study on the survival of insects on healthy and diseased plants seemed to indicate prolonged longevity on curly-top beets. However, his statistical analysis of the data showed that the difference was not significant.

KENNEDY (1951) found an increased longevity of aphids on virus-infected plants. It seemed that diseased plants provided a more favorable diet for the insect vectors.

A difficulty in evaluating these earlier observations and experiments lies in the fact that insects were always tested on plants which, with or without virus infection, provided adequate food for the tested animals. A different approach was therefore devised, using plants which under ordinary conditions were completely unsuitable for the animals.

Most leafhopper vectors of plant viruses can live on a wide range of food plants (OMAN, 1949). Examples of most omnivorous are *C. tenellus*, a vector of sugar-beet curly top, and *Macrosteles fascifrons* STÅL, a vector of aster yellows virus. An example of extreme limitation in the diet, on the other hand, is found in the two known insect vectors of corn stunt virus, *Dalbulus maidis* (DE LONG &

[1] Accepted for publication 4 August 1958.
[2] This work has been supported, in part, by Research Grant number E–1537 from the U.S. Public Health Service, National Institute of Allergy and Infectious Diseases.

WOLC.) and *D. elimatus* (BALL). These leafhoppers, as far as is known, are limited to only two food plants, *Zea mays*, the cultivated maize, and *Euchlaena mexicana* (teosinte), a wild grass believed to be an ancestor of maize. Numerous other plants were tested as possible sources of food for corn leafhoppers by KUNKEL (1948), NIEDERHAUSER & CERVANTES (1950, and personal communication) and by BARNES (1954). Although on *Calendula officinalis* adult *D. maidis* sometimes survive for four or five weeks and even deposit eggs (KUNKEL, 1948), nymphs hatched from these eggs die within a day unless removed to maize plants. Because of their host specificity, corn leafhoppers were chosen for our experiments. A preliminary account of the surprising findings and about the striking differences in survival of insects, found in these tests, has been published (MARAMOROSCH, 1958).

MATERIALS AND METHODS

Stock colonies of *D. maidis* and *D. elimatus* were maintained in an insectary on maize plants and adults and nymphs were available for experiments throughout the year. Standard techniques used for transmission of insects and for virus inoculations have been described in other reports (MARAMOROSCH, 1951, 1953). The eastern strain of aster yellows virus, *Chlorogenus callistephi* var. *callistephi* was employed. China asters, *Callistephus chinensis*, of the variety Shell Pink, used in all tests, were grown from commercial seed and transplanted into pots of various sizes.

EXPERIMENTS AND RESULTS

Survival studies

a. Adult leafhoppers

Eighty young adult *D. maidis* from stock were caged on two severely diseased China aster plants; 80 insects from the same stock were confined to two healthy aster plants; a third group of 80 adults were kept on two maize plants. After 24 hours 45 insects on the healthy aster plants were dead. On the morning of the fourth day no survivors were found on the healthy asters. On the diseased asters, on the other hand, all 80 insects were accounted for on the fourth day. Prolonged survival was then tested and it was found that on the 42nd day of the test, 63 insects were still alive on diseased asters, as compared to 68 on maize. In these tests it was necessary to transfer controls on maize every 10 days to avoid overcrowding by their progeny, while leafhoppers on diseased asters were transferred only once, after four weeks.

b. Nymphs

The surprisingly high survival rate of adult corn leafhoppers on yellows asters prompted tests with nymphs which, according to observations on Calendula plants, were even more restricted in their food requirements. Fifty second-instar nymphs were caged on healthy aster plants, 50 on yellows asters, and 50 on maize plants. All nymphs on healthy asters were found dead after 48 hours. On diseased plants nymphs survived almost as well as on maize. They molted normally, as did the controls, and eventually most became adults. After 25 days, there were 49 adults on maize, and 42 adults on the yellows asters. Six nymphs, however, were still in the third instar stage and remained so until they died three weeks later. As no inhibition of molting had ever been noticed during nine years of *D. maidis* breeding, the finding of an inability to molt was further studied.

One hundred second-instar nymphs were caged on diseased aster plants. After four weeks all but 16 insects became adults. The "retarded" nymphs, most in the third instar stage, were caged on a healthy maize plant. Rapid molting took place and 11 adults were obtained within a week. Five nymphs died in the fifth instar stage. Thus approximately 15% of corn leafhopper nymphs are unable to molt on diseased asters. Perhaps the new diet affects the synthesis of hormones in some, but not all, nymphs. The "retarded" nymphs retain their capacity to molt and do so when their natural food supply becomes available.

Breeding tests

Yellows asters on which adult male and female leafhoppers were caged for one month, were maintained for four additional weeks in the insectary to find whether eggs were deposited and whether nymphs would hatch. No progeny was obtained on yellows asters.

Were the physical conditions of the yellows aster plants unsuited for the depositing of corn leafhopper eggs, or did the new diet discourage mating? Two tests were made to find whether mating was curtailed or inhibited. In the first experiment five virgin females and five males were kept on yellows asters for three weeks. Then the males were discarded and the females confined for a single day to a maize plant. A total of 17 nymphs hatched, indicating that mating took place on yellows asters (*D. maidis* does not reproduce parthenogenetically).

In the second experiment, 10 females and 12 males, obtained from nymphs that molted on yellows asters, were kept for three weeks on diseased asters. Afterwards the females were caged for one day on a maize plant and discarded. Controls consisted of 10 females of comparable age, hatched, maintained and mated to 10 males on maize plants. A total of 22 nymphs were obtained from the aster-fed group, while the controls had 51 nymphs from eggs deposited within a single day.

In another test, 25 impregnated and already ovipositing females from maize plants were confined for two weeks to yellows asters. Although large numbers of nymphs hatched on maize previously colonized by these insects, none hatched on asters. This indicated that diseased aster plants, while providing an adequate diet for the survival of adult and nymphal stages were not adequate for breeding purposes. The corn leafhoppers were unable to have progeny on yellows asters under the conditions of the experiment. Although mating occurred, the insects were less prolific than were their counterparts on maize.

"Trained" insects

a. Adults

Twenty adult leafhoppers, maintained on yellows asters for five weeks, were transferred to healthy aster plants. After seven days 19 insects were still alive. Ten days later 14 survivors were accounted for. This surprising finding led to a number of tests with young adults as well as with nymphs, to determine the effect of "training" and the retention of the acquired ability to survive on healthy aster plants.

Did the feeding on yellows asters provide a chemical factor necessary for the survival of the insects and absent from healthy plants? If so, long maintenance on healthy aster plants should result in the depletion of this factor. It would also be expected that such a factor, acquired by feeding, would be depleted more rapidly after shorter feeding on yellows asters than after prolonged feeding. Several tests were made to gain insight into this matter.

Twenty five adult corn leafhoppers were confined to a yellows aster plant for seven days, then transferred to a healthy plant. Ten days later all the insects were alive. As controls 25 adults from stock maintained on maize were placed for 10 days on a healthy aster plant. All were dead on the fifth day. The 25 survivors were transferred from the healthy aster plant to maize plants for five days and later returned to healthy asters. This was done to find whether the acquired ability to feed on healthy asters would be retained or lost during the intervening feeding period on maize. It was found that the insects retained their acquired ability in spite of their intermittent maize diet: 15 insects were still alive on healthy aster plants after seven days, that is, on the 29th day of the test.

In another test the "interrupted training", that is, the intermittent feeding period on maize, was increased to 14 days. Nevertheless, the leafhoppers retained their acquired ability to feed on healthy aster plants. This test began with 25 insects, kept for seven days on a diseased aster plant, for 14 days on maize, and later on healthy asters. Twelve of 19 survivors transferred to healthy asters were still alive on the 31st day of the test. There was no evidence of a loss of the ability to survive on healthy aster plants.

b. "Trained" nymphs

Training tests of adults, that is, conditioning of the adults to feeding on healthy aster plants, was followed by similar tests with nymphs. Fifty second-instar and third-instar nymphs were confined to yellows asters. After seven days 38 surviving nymphs were transferred to maize for one week. On the 14th day 11 nymphs and 24 adults were separately caged on two healthy aster plants. The survival of adults was comparable to the survival in earlier tests in which the insects had acquired as adults the ability to feed on healthy plants. Twenty insects were alive on the 24th day. The survival of nymphs was poor, and only one adult and one nymph were found on the 24th day. This test showed that the conditioning effect ("training") persisted through the molting.

Virus retention

While the retention of a nutritional factor seemed an unlikely explanation for the acquired ability to feed on healthy aster plants, the retention of aster yellows virus by corn leafhoppers was given special attention. It has been known for many years that non-vector insects may acquire and retain plant viruses (BENNETT & WALLACE, 1938; SMITH, 1941; MARAMOROSCH, 1952). Aster yellows virus has been shown to be acquired by corn leafhoppers in short feeding periods. It has been recovered from the hemolymph of these non-vectors after 14, but not after seven days. Also, the virus has been retained through the molts. Could the conditioning of corn leafhoppers be connected with the acquisition of aster yellows virus? This virus causes in its insect host, the vector *M. fascifrons*, a chronic disease in which cells of a metabolic organ (fat body) are seriously affected (LITTAU & MARAMOROSCH, 1958). It seemed therefore possible that the virus could also exert pronounced effects on corn leafhoppers. Studies were undertaken to test this notion. Cytological studies, made by LITTAU, so far have revealed no changes in corn leafhopper fat bodies. These tests are being continued.

Several other approaches were available. The virus could be inactivated by heat treatments (KUNKEL, 1937), but heat treatment might also destroy other factors. Heat-inactivation tests are now in progress. Virus could be introduced from insect hemolymph by injection. Such tests, which eliminate feeding on diseased aster plants, at the same time introducing the aster yellows virus, are also in progress. The simplest approach, the acquisition of aster yellows virus by corn leafhoppers feeding not on diseased asters but on some other host of this virus, was tried. Thirty adult corn leafhoppers were confined on Calendula plants infected with aster yellows virus. After seven days 26 survivors were caged on healthy aster plants. Only two insects were alive on the third day. Although the result seemed to eliminate the virus as the cause of the conditioning effect, it still had to be ascertained that feeding on Calendula was comparable to feeding on yellows asters; previous data (MARAMOROSCH, 1952) on acquisition of virus were obtained by feeding *D. maidis* exclusively on asters. To check whether corn leafhoppers acquire the virus while feeding on diseased Calendula, the standard injection technique (STOREY, 1933; BLACK, 1941) was applied. Ten corn leafhoppers, maintained for 10 days on yellows Calendula and for an additional eight days on maize, were used to prepare an inoculum. Mechanical transmission was successful to *M. fascifrons;* this indicated that feeding on Calendula permitted the acquisition of the virus.

Although the results of the Calendula feeding suggest that the acquired ability of corn leafhoppers to survive on healthy aster plants was not caused by the acquisition and retention of aster yellows virus, the possibility of viral interaction could not be excluded entirely. Feeding tests on other hosts of the aster yellows virus, as well as heat inactivation and mechanical virus transmission, now in progress, should provide additional clarification.

Virus transmission tests

The leafhopper *D. maidis* belongs to the same family, subfamily and tribe as the common vector of aster yellows virus *M. fascifrons*. Although attempts were made in previous years to test the corn leafhopper as a potential vector of aster yellows virus, these earlier tests were limited to very short test feeding periods on asters. After the discovery of the conditioning effect, transmission attempts with "trained" insects were made. No transmission of aster yellows virus to China asters was obtained.

Following STOREY's classical experiments with *Cicadulina mbila* (STOREY, 1933) 20 trained corn leafhoppers were punctured with insect pins 20 days after they begun feeding on yellows asters. Subsequently the insects were tested on aster seedlings. No transmission was achieved.

Tests with D. elimatus

The second vector of corn stunt virus, *D. elimatus*, has a similarly limited and restricted diet as *D. maidis*. Tests with *D. elimatus* followed the same pattern as tests described above, and will therefore not be described in detail. The training results were similar with adults, but less successful with nymphs. It appears that

D. elimatus nymphs are even more particular than nymphs of *D. maidis* in their restriction to a maize diet. Transmission tests with punctured insects gave negative results.

Breeding attempts on Calendula

In view of KUNKEL's finding that *D. maidis* adults will often survive for long periods of time on healthy Calendula plants and deposit eggs, from which viable first-instar nymphs can be obtained, an attempt was made to breed corn leafhoppers on yellows Calendula. Against expectations, these breeding attempts failed completely. Even the survival of adults on diseased Calendula was unsatisfactory. These tests will be continued.

Conditioning on plants infected with other viruses

In a limited number of tests, adult *D. maidis* and *D. elimatus* were confined to crimson clover (*T. incarnatum*) plants infected with wound tumor and to sugar beets with curly top. Neither of these diseased plants provided adequate conditions for the survival of corn leafhoppers.

DISCUSSION

The finding that yellows asters provide an acceptable diet for nymphs and adults of corn leafhoppers, while healthy plants are unsuitable, open new experimental possibilities in an important field, which has received little attention in the past. Why are certain species of insects highly specific in their food requirements? What decides that maize plants provide a suitable source of food for *D. maidis*, while almost all other plants are unsuitable? What change from the healthy China aster to yellows aster plant makes it become suitable for corn leafhoppers, and what is the mechanism of the "memory", which permits feeding on healthy China asters? Further investigations on the adaptation of corn leafhoppers may answer these and many other questions and elucidate the fascinating problems of food specificity.

The conditioning effect, by which the insects become trained through a diet on yellows asters to accept healthy plants, is of great interest and significance. Probably, healthy aster plants are not poisonous, but only repulsive to corn leafhoppers. Presumably, the insects starve to death rather than try to feed on such plants. When the same insects are confined to yellows asters, they either become attracted to them or at least are no longer repelled. In consequence, they learn to accept the new, strange food plant. Moreover, the individuals that have learned to feed on yellows asters retain this acquired ability even through molts and over long periods spent on their conventional food plant.

We do not know why corn leafhoppers become conditioned by feeding on aster plants infected with the aster yellows virus. The virus might exert control over the plant-insect relationship by a variety of mechanisms. Virus acquired through feeding on diseased plants is retained in the body of the non-vector insects. The virus might act directly on some organs and tissues of the non-vector, as it does on the cells of the fat body of the vector, causing as yet unnoticed cytopathogenic changes. It is conceivable that viral multiplication in cells of corn leafhoppers may result in the induction of an enzyme. If an adaptation process (to feed on new hosts) is ordinarily inhibited by a chemical substance, the induced enzyme might destroy the inhibitor. The induction of a new enzyme might also give the corn leafhopper the ability to utilize the phloem sap of aster plants, which is otherwise unpalatable.

The virus might also act on the plant-insect interrelationship by conversion of the plant host. It might, on the one hand, cause the production of a substance essential for the survival of corn leafhoppers, or the removal of an inhibitor.

The virus could, on the other hand, influence the physicochemical nature of plant cells affecting the structure and composition of the cell walls. Such a change could turn an unsuitable plant into a suitable host for the insect.

The conditioning is of interest from a theoretical as well as a practical point of view. The training of corn leafhoppers provides a system for the study of the development of the conditioning phenomenon as related to the length of the training period. It also permits the study of the persistence of the acquired adaptation in relation to the length of training. Finally, conditioning can be studied in relation to various stages of infection of the plant.

From a practical standpoint, the phenomenon of conditioning through feeding on virus-diseased plants is of considerable importance to agriculture. Although aster yellows virus is a serious disease of many plants in over 50 families (KUNKEL, 1931), it may exert a beneficial effect as far as the survival of corn leafhoppers is concerned. For these animals, the altered diet may mean the difference between death and survival during periods when corn plants are unavailable. It is conceivable that more such "beneficial" relationships between arthropods and virus-diseased plants will be found when a search for them is made in nature or in the laboratory. Invasions of new host plants, particularly of important crops, by species of insects not previously known to feed on such plants, might be favored by such conditioning. This, in turn, could result in the wider spread of viruses, particularly of those that can multiply both in plants and in insect vectors.

SUMMARY

Earlier reports in the literature on increased longevity of insect vectors feeding on virus-infected plants were supported by new findings. Instead of using virus-free and virus-infected plants on which a certain vector ordinarily feeds, an unsuitable food plant was chosen. *Dalbulus maidis* leafhoppers could not be maintained on healthy China aster (*Callistephus chinensis*) plants. However, on asters severely infected with aster yellows virus, survival of adults and nymphs was nearly the same as on maize, their proper food plant. Adults and nymphs maintained on diseased asters, became conditioned to the feeding on the new host and could later be maintained also on healthy asters. No progeny was obtained on aster plants. It could not be established whether the beneficial effect of feeding on diseased plants was caused by acquisition of virus. Although retained by corn leafhoppers, aster yellows virus was not transmitted by them to aster plants. The prolonged survival of corn leafhoppers on healthy aster plants after a feeding period on yellows asters, is a conditioning phenomenon. The possible mechanisms involved in this conditioning, and the usefulness of the system for further studies are discussed. The significance of the findings is presented in relation to the invasion and spread in nature of insect pests and virus vectors.

SAMENVATTING

Resultaten van verschillende proeven geven steun aan het in de literatuur vermelde verschijnsel, dat insekten-vectoren op viruszieke planten langer in leven blijven, dan op gezonde planten. In deze proeven werden ongeschikte voedselplanten gekozen, in plaats van gezonde en viruszieke planten, waarop de vector zich normaliter voedt.

Individuen van de cicade-soort *Dalbulus maidis* konden zich niet handhaven op gezonde asterplanten (*Callistephus chinensis*). Op asterplanten, die hevig door aster yellows virus waren aangetast, konden de larven en imagines echter even goed leven als op maïs, hun eigenlijke voedselplant. Zij gewenden aan deze nieuwe voedselplant en konden zich later ook op gezonde asters handhaven. De cicaden plantten zich op asters evenwel niet voort.

Er kon niet worden vastgesteld, of het gunstige effect van de viruszieke planten op de cicaden werd veroorzaakt door het opnemen van virus.

Hoewel de dieren het virus opnamen en het bij zich hielden, waren zij niet in staat het op gezonde asterplanten over te brengen.

De mechanismen, die verantwoordelijk zouden kunnen zijn voor het gewennen aan de nieuwe voedselplant en de mogelijkheid van de bestudering van de problemen, die daarmee verband houden, worden besproken. Er wordt gewezen op de betekenis van het verschijnsel van gewenning voor de verbreiding van insektenplagen en virusvectoren.

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