A LEAFHOPPER TRANSMITTED CLOVER DISEASE IN THE OTTAWA AREA

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Abstract

Ladino clover (Trifolium repens) and alsike clover (Trifolium hybridum) plants exposed to leafhoppers, Aphrodes bicinctus, collected from a field at the Central Experimental Farm, Ottawa, Ontario, developed chlorosis, reddening of leaf margins, adventitious growth at the nodes, witches' broom, and smaller than normal or rudimentary leaves. Flowers were reduced in number and size but appeared to be functional. The percentage of field-collected leafhoppers that induced the disease was increased by feeding the insects on abnormal appearing alsike clover plants obtained from the same field. This together with the time required for symptom appearance indicate that the disease is caused by a transmissible agent. Although most of the individual symptoms have been observed on clover plants infected with various other diseases, the combination of symptoms suggests that this disease may not have been described before,

Introduction

The only causal agent of plant disease known to be transmitted by the leafhopper Aphrodes bicinctus (Schrank) in North America is that causing clover phyllody **(2).** Since clover phyllody has not been found in the Ottawa area, it was expected that field-collected insects of this species would be free from plant disease inducing agents and could be used in transmission studies on clover phyllody. Accordingly, adult & bicinctus leafhoppers were collected from a field of grass and volunteer clover at the Central Experimental Farm, Ottawa, and used in an experiment. A group of 25 of these field-collected insects was maintained as a control and was transferred weekly to healthy ladino clover (Trifolium repens L.) or alsike clover (Trifolium hybridum L.) plants. All five plants on which these insects were caged developed symptoms that did not resemble those of clover phyllody.

Symptoms

The following symptoms were observed on plants infected in the greenhouse. The first signs of infection appeared about a month after inoculation in the form of a mild chlorosis of new growth in both ladino and alsike clovers. Plants were generally stunted and leaf margins were reddish or chlorotic (Figs. 1 and *2).* Old leaves were cupped downward toward the abaxial side, slightly bronze in color and died prematurely. In ladino clover clusters of small short-petioled leaves with downward cupped chlorotic margins developed at the nodes of the creeping stems (Fig. **3).** The single long-petioled trifoliolate leaf that normally develops at the node was often missing. In some plants, creeping stems were absent and numerous small leaves with upright petioles developed, giving the plant a witches'-broom appearance (Fig. 4). Some of the new leaves were slow to unfold and were curved at the ends *so* that they took on a "pin wheel" appearance. Later developing leaves were rudimentary and consisted of **3** short stubs in place of the normal **3** leaflets (Fig. 5). In alsike clover the crown was thickened and the upright sterns produced clusters of adventitious growth at the nodes. These clusters consisted of short-petioled leaves that were reduced in size and had chlorotic margins (Fig. 6). Often the upright stems did not develop and the infected plants took on a witches'-broom appearance (Fig. 7). Although flowers in both species of clover were reduced in number and size, they appeared to be functional.

A search of the area from which the leafhoppexs were collected did not reveal clover plants with symptoms typical of those observed on plants infected in the greenhouse. However, several abnormal alsike clover plants were found which had dense crowns of small, short-petioled leaves and three or four upright stems. The foliage of these plants was slightly bronze colored.

Transmission of causal agent

Because most groups of leafhoppers collected from the field were already capable of inducing the disease, the possibility existed that the disease was the result of toxins injected by the insects while feeding rather than a transmissible agent. Although no "healthy" insects were available, it was felt that evidence for or against a transmissible agent being involved could be obtained by exposing field-collected insects to plants with symptoms and determining if the percentage of insects inducing symptoms would increase. Two experiments were conducted using abnormal alsike clover from the field as source plants.

In the first experiment, three groups (27 insects per group) of field-collected leafhoppers were caged

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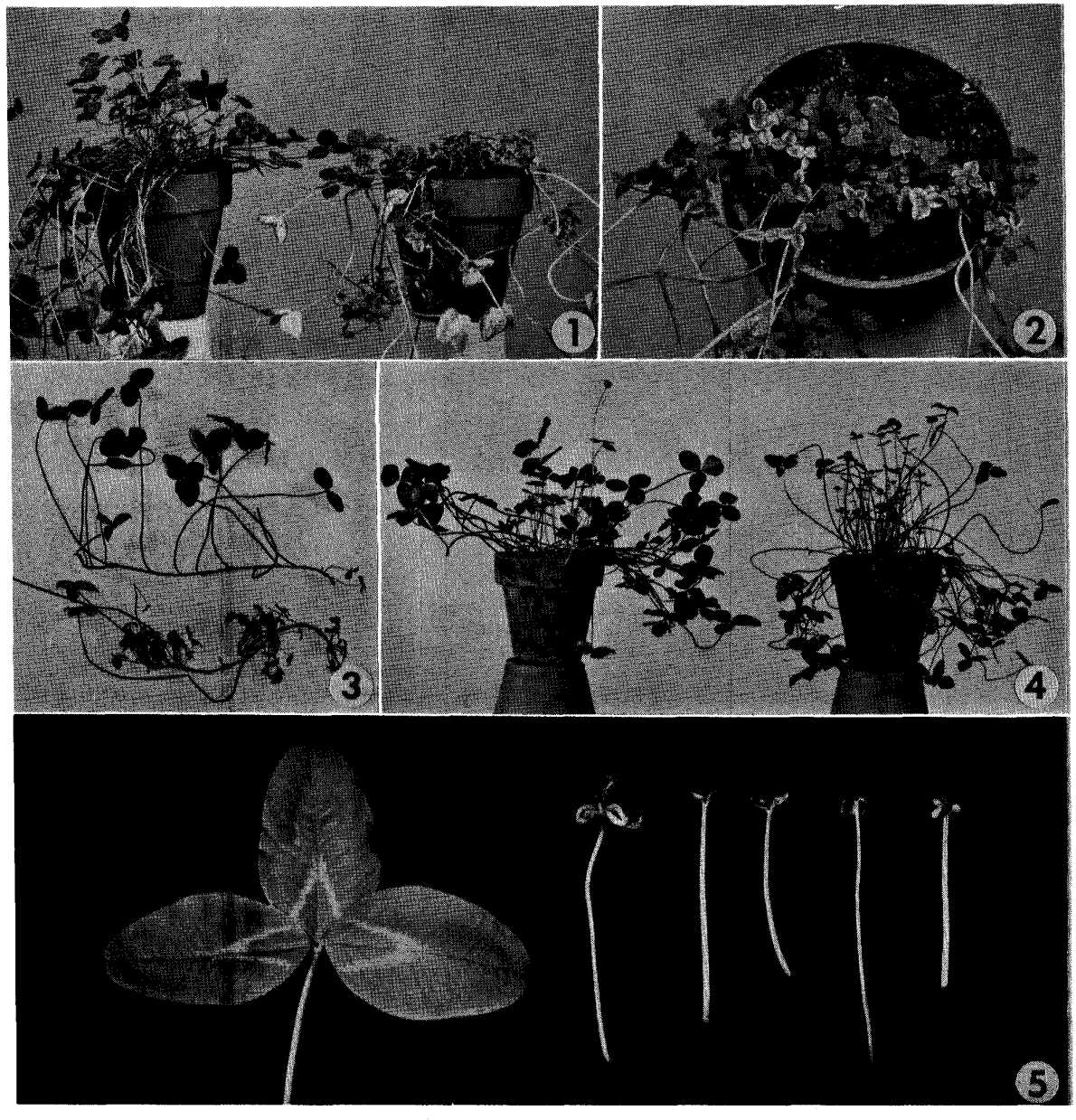
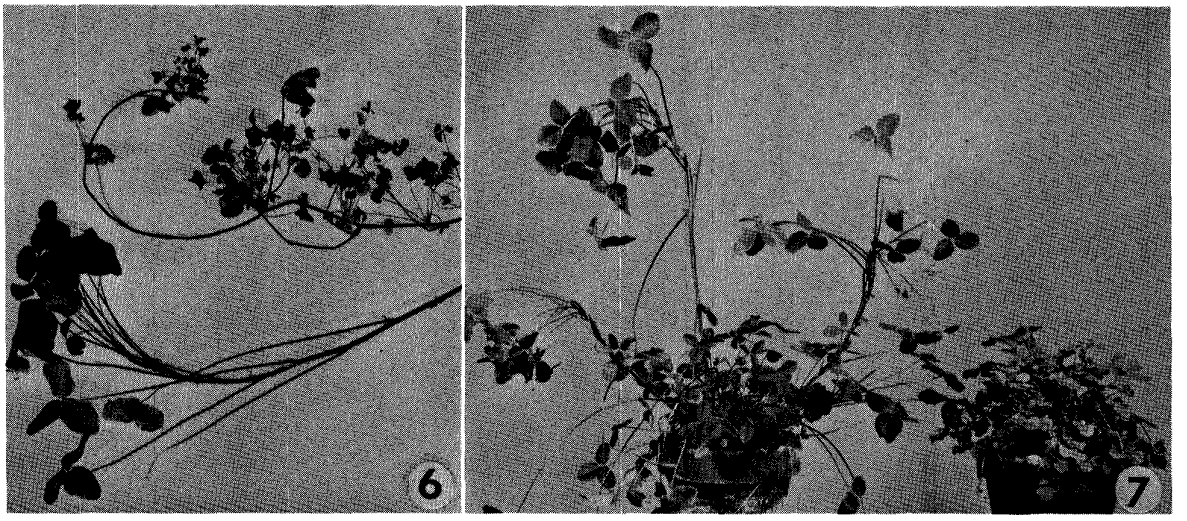


Figures 1–5. Symptoms of an unidentified leafhopper-borne disease on ladino clover (*Trifolium repens*). Figure 1 (right) and Figure 2. Plants stunted and leaves with chlorotic margins; Figure 1 (left) healthy plant. Figure 3. Lower, infected plant showing creeping stem with clusters of small short-petioled leaves at the nodes; upper, healthy. Figure 4. Right, infected plant locking creeping stems and showing witches' broom. Left, healthy. Figure 5. Right, Infected leaves, showing reduced size, rudimentary leaflets, and pin-wheel symptoms. Left, healthy leaf.

on a healthy ladino clover plant. Each group of exposed and control insects was then transferred weekly to a healthy ladino clover plant. Sixty-two days from the start of the experiment, the 20 survivors from the exposed groups and the 9 survivors from the control group were caged singly for two weeks on alsike clover seedlings.

Two of the exposed groups of leafhoppers induced symptoms on all healthy plants on which they



Figures 6 and 7. Symptoms of unidentified leafhopper-borne disease in alsike clover (*Trifolium hybridum*). Figure 6. Upper, infected stem with clusters of small, short-petioled leaves at nodes. Lower, healthy. Figure 7. Right, infected plant with no upright stems and showing witches'-broom symptom. Left, healthy.

were caged following exposure to the source plants. The third group produced symptoms for the first time on the second healthy plant (18-25 days from the start of the experiment) as well as all subsequent plants on which they were confined. The plant on which the control insects were caged for the first 11 days remained healthy but all subsequent plants developed symptoms. Eleven of the 20 (55%) exposed insects tested singly produced symptoms, while only 1 of the 9 (11%) control insects induced infection.

In the second experiment, 50 field-collected adult leafhoppers were caged for 7 days on each of 3 field-collected alsike clover plants: and as a control, 50 field-collected insects were caged on a healthy ladino clover plant. Both exposed and control insects were transferred weekly to healthy ladino clover plants. Twenty-eight days after the start of the experiment, all surviving insects were caged singly for 2 weeks on healthy ladino clover seedlings.

Each of the three groups of exposed leafhoppers induced symptoms on the first healthy plants on which they were confined (7-14 days from the start of the experiment) and on the two subsequently exposed plants. The control insects also produced symptoms on all plant on which they were caged. When tested singly, 10 of 42 (24%) of the exposed and 1 of 27 (4%) of the control insects caused infection.

Discussion

The results of both experiments showed that the number of field-collected A. bicinctus leafhoppers capable of inducing the disease could be increased by allowing them to feed on diseased clovers. Furthermore, symptoms on plants did not appear until 3 weeks or longer after the insects were removed. Both of these facts point to the conclusion that this clover disease is caused by a transmissible agent. Had a toxin been involved, symptoms should have appeared while the insects were still on the plant or shortly after the insects had been removed.

The symptoms produced and the fact that the causal agent is leafhopper transmitted suggest that this disease may belong to the "yellows" group. Recent findings (3) indicate that this group of diseases may not be caused by viruses as was originally supposed, but rather by Mycoplasma-like organisms. Further studies will be required *to* characterize the etiological agent involved in this disease.

Attempts to identify this disease on the basis of symptomatology have been unsuccessful. A direct comparison with witches' broom infected clover plants from Vancouver, B. C., revealed differences in leaf shape, size and color. Similarities with Australian legume little leaf were noted by Mr. J. W. Bowyer (personal communication) with the exception that little leaf results in phyllody. Foliar symptoms resembel those described for the parastolbur disease found in Europe (4), but again the flowers of parastolbur infected plants exhibit phyllody. Dr. L.M. Black (personal communication), on first observing the disease, remarked on its general similarity to clover club-leaf, a disease transmitted by Agallian leafhoppers (1). Closer examination, however, disclosed certain differences in leaf shape and coloration. Present indications are that this disease had not been described before.

However, since the present work was done with field-collected insects and source plants, one cannot rule out the possibility that more than one disease or causal agent is involved. It is hoped that the studies on vector–causal agent–host plant relationships now in progress will provide some of the answers.

Acknowledgments

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