INVESTIGATIONS OF CHRYSANTHEMUM VIRUSES IN CANADA
I. Chrysanthemum Rosette Virus[1]

W. G. Kemp[2]

Abstract

A virus disease of chrysanthemum, hitherto unreported in Canada, is described. The causal agent is considered to be identical with, or closely related to the Ivory Seagull rosette virus. It has been transmitted from symptomless plants of the variety Wilson's White to Blazing Gold by grafting and mechanical inoculation where it causes veinbanding, leaf distortion and dwarfing, and later, rosetting of the new growth. This virus has been detected in two additional florists' chrysanthemum varieties and in nine garden varieties.

Introduction

In 1959, during the routine graft-indexing of florists' chrysanthemum varieties to select plants free from stunt virus for further development and production, a commercial propagator in Southern Ontario observed unfamiliar symptoms in Blazing Gold which had been grafted to symptomless plants of the variety Wilson's White. The grafted plants that showed unusual symptoms suggestive of a virus disease and rooted cuttings from affected stock of this variety were submitted to the Research Laboratory, Vineland Station, Ontario for a diagnosis of the condition.

Only four virus diseases of Chrysanthemum ~~morifolium~~ Ramat. have been reported in Canada: aster yellows (6); spotted wilt (5); stunt (1); and stunt-mottle (7). None of the viruses that cause these diseases produce symptoms in the indicator variety Blazing Gold that are identical with those induced by the virus detected in plants of Wilson's White. Consequently, further investigation of this graft-transmissible disorder was conducted. This paper records the identity of the causative agent and presents additional data relative to its transmission and its occurrence,

Materials and Methods

Plants of the variety Wilson's White carrying the unknown virus (WWV), without symptoms, have been used as the source of the virus throughout this investigation. These stock plants were further assayed for the presence of other chrysanthemum viruses by a graft-inoculation method commonly used by specialist propagators to select virus-free material. Healthy scions of test

---

[1]Contribution No. 9, Research Laboratory, Research Branch, Canada Department of Agriculture, Vineland Station, Ontario,

[2]Plant Pathologist,

varieties approximately four inches long were cleft-grafted to succulent, two- to three-inch stem sections of the plants to be indexed, The grafts were then bound tightly with surgical latex strips, and immediately stuck in two-and-a-half-inch clay pots filled with a peat-perlite mixture to root. When rooted, these grafts were repotted in four-inch pots, staked, and grown to flowering. Either Bronze or Pink Mistletoe chrysanthemums were used to detect the stunt, aster yellows and mosaic viruses. The variety Blazing Gold was used to indicate the presence of both the flower distortion and the rosette viruses.

Sap inoculations to Nicotiana tabacum L. var. Harrow Velvet and to Petunia hybrida Vilm. were used to detect aspermy virus and to further help in the identification of the so-called mosaic viruses. Mechanical inoculations were made by macerating infected chrysanthemum leaf tissue in its own weight of distilled water or 0.1 M phosphate buffer at pH 7.0. Carborundum was dusted lightly over the foliage of the indicator plants prior to rubbing them gently with the forefinger moistened with inoculum.

Symptoms

WWV-infected Wilson's White rarely shows detectable disease symptoms which, even when present, are of uncertain diagnostic value because infected plants may be carrying other viruses as well. For the most part, this variety has remained symptomless or shown mild, transitory vein clearing during the 18-month period that the originally infected stock plants have been under surveillance. No abnormal flowers were observed nor were they reported by the propagator from which the material came.

In graft-inoculated Blazing Gold, WWV initially produces pronounced veinbanding, leaf distortion and puckering in young plants. Later, growth is checked and subsequent development is rosetted. Stems of stunted plants show extremely short internodes and the rosetted foliage is noticeably dwarfed (Fig. 1 A-B). Symptomless regrowth of severely rosetted Blazing Gold sometimes occurs, particularly in the latter part of the summer. Flowers on the infected indicator variety are slightly misshapen. No distinctive symptoms occurred in three to five months on the graft-inoculated Bronze and Pink Mistletoe varieties.

Transmission

By grafting: The first successful transmission of WWV was observed in June, 1959, on four Blazing Gold scions that were top-grafted to four symptomless but different Wilson's White sources during the previous March.

Subsequently, in March, April, June, and August, 1960, a series of top-grafts were made using as stock, sources of the variety Wilson's White known to be infected with WWV and as scion, the varieties Blazing Gold and Pink Mistletoe. Twenty-four of the 30 Blazing Gold - Wilson's White graft combinations that were successfully rooted and grown, showed rosetted growth in two months. None of the scions of the 11 rooted Mistletoe-Wilson's White combinations showed symptoms in three to four months.

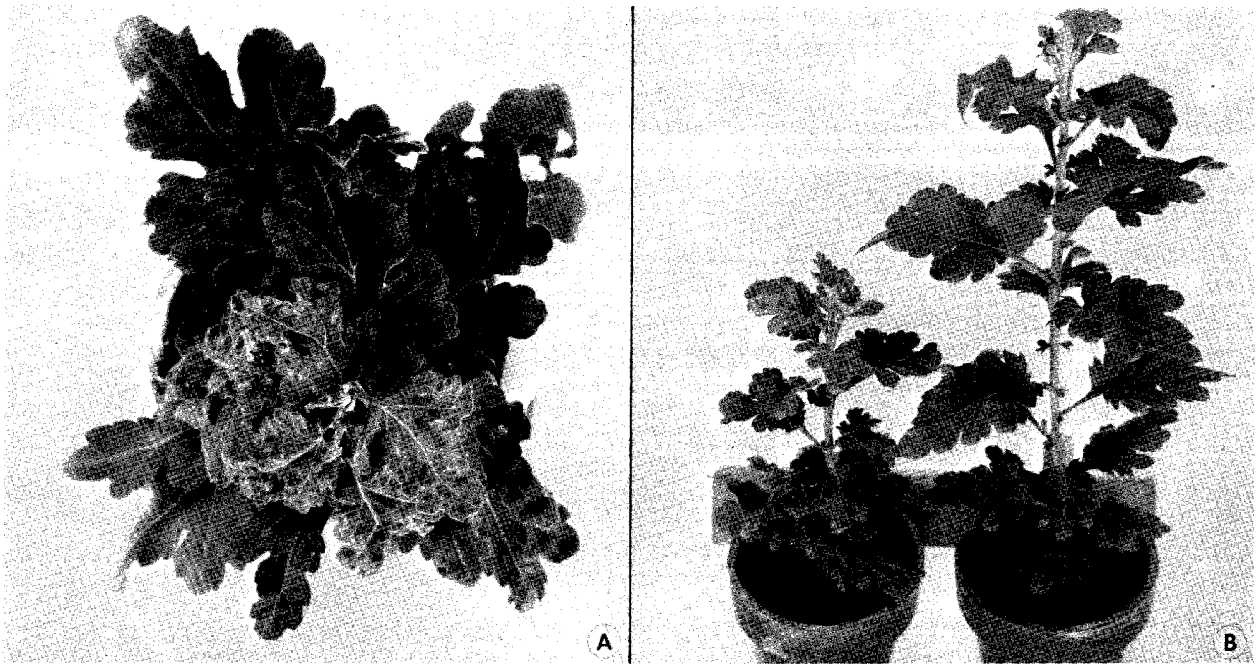

Fig. 1. Symptoms of chrysanthemum rosette virus.

A. Blazing Gold showing leaf distortion and rosetting three monthsafter graft-inoculation.

B. Left, Blazing Gold top-grafted to rosette infected Wilson's White.
Right, Blazing Gold top-grafted to rosette-free Wilson's White.

Transmission was detected on some of the Blazing Gold scions as early as five weeks after the March, 1960, grafts were made. Symptoms were apparent four weeks after the April graftings and six weeks after the June and August graft-inoculations. The initial symptoms appeared as a clearing of the larger veins followed by puckering and distortion of the youngest leaves. All of the infected scions later showed stunting, rosetting, and leaf dwarfing.

By sap: Attempts were made to effect transmission of the virus by mechanical inoculations with extracts from infected chrysanthemum plants. Sap inoculations in June, 1959, March, July, and August, 1960, failed to demonstrate virus transmission to petunia or tobacco seedlings from WWV-infected leaves in a six-week period. Leaf extracts from a few of the inoculated tobacco and petunia plants did not induce rosette symtoms in back-tests to Blazing Gold.

In July, August, and October, 1960, sap from infected Blazing Gold leaves was rubbed on the foliage of 10 young four-to six-inch plants of Blazing Gold and Pink Mistletoe. In seven to eight weeks after the July inoculation dates, two of the 10 Blazing Gold became infected. No additional plants of this series showed symptoms after three months. Neither indicator variety showed symptoms after four months following the August inoculations. In the October test, one of the 10 inoculated Blazing Gold plants became infected in five weeks; another showed visible symptoms during the sixth week, At that time, a six-inch terminal portion of the stem of each of the eight plants that were symptomless was removed with a sterile scalpel, Two of these topped plants became infected; the first two weeks later, the other in the third week.

No increase in the number of plants infected was obtained by extracting the sap from infected chrysanthemum leaves with 0. 1 M phosphate buffers adjusted to pH 5. 0, 6. 0, 7. 0 and 8. 0.

By insects: Apterous Myzus persicae Sulz. (Homoptera:Aphididae) failed to transmit the virus from infected Blazing Gold to healthy Blazing Gold. During the summer of 1960, nonviruliferous aphids were reared on healthy radish seedlings prior to starving them for from four to five hours on moist filter paper in a petri dish. They were then given an acquisition feed lasting from two to five minutes on detached leaves of infected Blazing Gold. Aphids were then transferred to each of 10 young, healthy Blazing Gold indicator plants and these plants were caged immediately. Twenty-four hours later, all of the cages were removed and the plants were sprayed with malathion to destroy the aphids. No transmission occurred in three months in either of the two tests conducted in July and August. Healthy control plants did not become infected following the transfer to them of Myzus persicae previously fed on healthy detached Blazing Gold leaves.

Two additional aphid species, Macrosiphoniella sanborni (Gill.) and Aphis sp., found to breed well on chrysanthemums, were used in other transmission tests. Single WWV-infected Blazing Gold plants, each previously infested with one of the aphid species, were caged with six healthy indicator

plants. The healthy plants were slowly colonized by alatae of each species from the infected plants. After a month, all of the plants were removed from each cage and sprayed with malathion. At the time of their removal from the cages, no symptoms were apparent on the original healthy Blazing Gold nor were they observed on these same plants three months later.

Identity of the Virus

WWV produces certain symptoms in the chrysanthemum indicator variety Blazing Gold in common with the flower distortion virus, the aster yellows virus, and the rosette viruses, Brierley and Smith (3) found that Blazing Gold showed a characteristic rosetted growth when infected with flower distortion virus at all seasons and that this variety responded to inoculation with aster yellows virus in a similar manner in winter but not in summer.

However, distinct differences exist between WWV and either of the other two viruses that exclude the possibility of any relationship. Flower distortion virus is not transferred mechanically whereas WWV is; it also induces symptoms in Blazing Gold a month after WWV can be detected in this variety. Furthermore, the distortion virus is reported to be lethal to Blazing Gold between five and seven months after graft-inoculation (3). No lethal effects have been noted in Blazing Gold-Wilson's White graft combinations after an eight month period. The failure of aster yellows virus to transfer mechanically and the fact that it usually induces green flowers in infected varieties indicates that it is not similar to WWV, which can be transferred by sap inoculations and has not produced green flowers in either Blazing Gold or Wilson's White.

WWV, however, appears to be identical with, or closely related to, Brierley and Smith's (4)Ivory Seagull rosette virus, It is one of two additional viruses known to occur in the United States that induces a rosetting effect in Blazing Gold at all seasons except in summer. Both WWV and Ivory Seagull rosette virus are sap-transmissible with difficulty and produce symptoms in Blazing Gold in a month. They have failed to infect petunia following mechanical inoculation and neither has been transferred by Myzus persicae from infected Wilson's White to Blazing Gold in the non-persistent manner. On the other hand, Ivory Seagull rosette virus causes veinbanding with leaf distortion in Golden Mistletoe in from three to four months and rosetting in seven months. WWV has failed to induce visible symptoms in this variety. This, however, may simply be due to the fact that the grafted and mechanically-inoculated plants were under observation for only three months.

The fact that Yellow Rayonante rosette virus, considered by Brierley and Smith (4) to be distinct from Ivory Seagull rosette virus, produces veinbanding, interveinal mosaic, leaf dwarfing, and distortion in Mistletoe one month after grafting and leaf necrosis in two months precludes the possibility of a relationship between it and WWV.

Because of the low percentage of successful mechanical transfers of WWV to the susceptible variety, Blazing Gold, experiments have not as yet been conducted to determine the physical properties of this virus.

Incidence

The distribution of the rosette virus in the large numbers of florists' chrysanthemum varieties that are available commercially in Ontario and its prevalence in an infected variety have not been investigated. Its identification by symptom expression alone is probably inaccurate. The problem of diagnosis is complicated by the almost total absence of symptoms in the leaves of some varieties and by mixed virus infections. Graft-inoculation to Blazing Gold followed by back-inoculation with sap from rosetted plants to the same variety is the most reliable detection method. By this method, the virus has been found to occur alone in Wilson's White and with other viruses in Mason's Bronze and Garnet King as well as in the garden varieties Champion Cushion, Jess Williams, Joan Helen, Lavender Lady, Masquerade, Orsona, Powder-puff, Remembrance, and Reflection.

Discussion

Rosette has not been previously reported in chrysanthemums in Canada and its importance to the crop is difficult to assess. Brierley and Smith **(2, 4)**, who first described the disease in the variety Ivory Seagull in 1950 in the United States, reported that because no specific test was known for detecting this rosette virus. in the presence of more infectious viruses, its distribution and and prevalence remain obscure. From the experimental evidence obtained to date, it is unlikely that this disease will be a serious economic factor in chrysanthemum production because of its mild effect on varieties naturally infected with the virus and because of the difficulty of sap transmission. However, though the virus and certain affected varieties appear compatible and the plants produce vigorous growth and quality flowers, the possibility exists that other valuable varieties will be less tolerant and may react in a manner similar to the indicator variety Blazing Gold, or even more drastically.

Acknowledgments

The author gratefully acknowledges the identification of the aphids used in the transmission studies by Mr. W. R. Richards, Canada Department of Agriculture, Research Branch, Entomology Research Institute, Ottawa, Ontario, and the technical assistance of Mrs. **P.** Heald and Mr. W. Haigh. Thanks are due to Atkin's Flowers Ltd., Leamington, Ontario for providing certain chrysanthemum varieties.

Literature Cited

1. BERKELEY, G. H. 1948. in Can. Plant Dis. Survey. 27th Ann. Rep't. 105-106.
2. BRIERLEY, PHILIP and F. F. SMITH. 1951. Survey of virus diseases. in chrysanthemums. Plant Dis. Reptr. 35: 524-526.
3. BRIERLEY, PHILIP and F. F. SMITH. 1957. Symptoms of chrysanthemum distortion, dodder transmission of the virus, and heat cure of infected plants. Phytopathology 47: 448-450.
4. BRIERLEY, PHILIP and F, F. SMITH, 1958. Some characteristics of eight mosaic and two rosette viruses of chrysanthemum. Plant Dis. Reptr. 42: 752-763,
5, SANFORD, G. B. 1942. in Can, Plant Dis. Survey. 21st. Ann. Rept. 89.
6. SAVILE, D. B. O. 1945. in Can. Plant Dis. Survey. 24th Ann. Rept. 107,
7. WELSH, M. F. 1948. Stunt-mottle virus disease of chrysanthemum. Sci. Agr. 28: 422.

CANADA AGRICULTURE RESEARCH LABORATORY,
VINELAND STATION, ONT.