Effect of Tobacco Ringspot Virus on Yield, Persistence, Flowering, and Seed Set of Lotus corniculatus

S. A. Ostazeski, P. R. Henson, and C. S. Garrison

Research Plant Pathologist and Research Agronomists, respectively, Crops Research Division, ARS, USDA, Beltsville, Maryland 20705.

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ABSTRACT

The effect of chronic tobacco ringspot virus (TRSV) infection was measured in three clones of birdsfoot trefoil. In greenhouse tests, measurements were made on virus-infected as well as virus-free clones on number and length of roots produced on vegetative cuttings and dry weight yields taken in pot culture. Field plantings were used to evaluate vigor, persistence, umbels per plant, pods per umbel, seed per pod, and weight per 100 seed. Analyses of variance showed clonal differences to be highly significant (.01) in all tests. Virus effects were highly significant, or became so with time, in all tests. Clone × virus interactions were significant in all measurements except number of roots produced, dry wt of top growth, and wt per 100 seed. These observations emphasize that TRSV-infected trefoil plants, though symptomless, are deleteriously affected by the virus, and that clonal differences in reaction to the virus do exist. Phytopathology 60:1753-1755.

Additional key words: forage crops, legumes, symptomless virus reservoirs.

Stands of forage legumes often thrive 2 to 4 years before productivity declines. Examination of older fields often revea's a high percentage of virus-infected plants. Kreitlow (4) and Kreitlow & Price (7) suggested that infected Ladino clover plants become weakened and are more susceptible to drought and winter injury than healthy plants. Alfalfa mosaic and bean yellow mosaic viruses, alone and in a mixture, also affected chemical composition and reduced forage yield, flowering, and seed production of Ladino white clover (5, 6). Roberts (9) showed that Ladino clover plants infected with alfalfa mosaic virus are more susceptible to cold damage than virus-free plants. In field experiments, Goth & Wilcoxson (3) showed that bean yellow mosaic virus infection can reduce stands of red clover as much as 100% under seasonal temp extremes characteristic of Minnesota. They found flower development on virus-free plants to be 2- to 4-fold that of virus-infected plants. Seed production on infected plants was one-tenth that of healthy plants.

Birdsfoot trefoil is a natural host of tobacco ringspot virus (TRSV). After having reported TRSV in birdsfoot trefoil at Beltsville, Md. (8), we recovered the virus from plants grown at Blacksburg, Va., and Columbia, Mo. Typical local lesions followed by a ring and line pattern symptom are produced on birdsfoot trefoil inoculated with TRSV. Like many other plant species, birdsfoot trefoil recovers from acute infection and passes into a symptomless, but viruliferous, chronic phase. Symptoms of virus infection in forage legumes are known to be masked at times by environmental conditions. In no other forage species, however, does a combination of small leaflet size and fleeting symptom expression make field diagnosis more difficult than TRSV in birdsfoot trefoil. The present study was undertaken to determine the influence of chronic TRSV infection upon parameters of vegetative and floral growth.

MATERIALS AND METHODS.—We compared virus-free and virus-infected plants of clones I, II, and III. A set of virus-free plants was inoculated with the 2B-59 strain of TRSV (8). A comparable set of plants of the same clones was maintained separately, free of TRSV. Stem cuttings were taken from all test plants only after all symptoms on inoculated plants had disappeared. All cuttings were rooted in tap water in test tubes using five two-node cuttings/tube.

RESULTS.—*Greenhouse tests.*—Two separate, completely randomized experiments were conducted to determine the effect of TRSV on rooting of propagules. Roots of each cutting were counted, and the longest was measured after 14 days in tap water in Experiment 1 and after 20 days in Experiment 2. At the conclusion of the first experiment, randomly selected rooted cuttings were taken from each entry and planted singly in sterilized soil in 10-cm clay pots. Pots were arranged in a randomized complete block design with six entries and 11 replications. The top growth was cut, dried, weighed, and recorded in g per plant for three harvests taken 55, 82, and 160 days after planting.

In Experiment 1 (Fig. 1-2), virus infection did not significantly affect the number of roots produced on the stem cuttings in 14 days; however, virus infection significantly reduced the length of the longest root. Differences in the number of roots as well as the clone × virus interaction for the length of the longest root were highly significant at 20 days (Experiment 2).

The first top growth yields taken from clones 55 days after establishment were significantly different (Fig. 3). Although there was no significant difference due to virus effects, there was a significant clone × virus interaction. Yields taken on the second and third harvests (82 to 160 days after planting, respectively) showed highly significant clone and virus effects (Fig. 3). The clone × virus interaction was not significant in the second and third harvests. Yield differences within clones for the third harvest indicates that virus effects are exaggerated with a passage of time.

Field tests.—In March 1966, cuttings from healthy and TRSV-infected plants of the three clones were rooted in water and planted in sterilized soil in peat pots. Losses during establishment in peat pots in the greenhouse reduced the number of some entries (lowest,

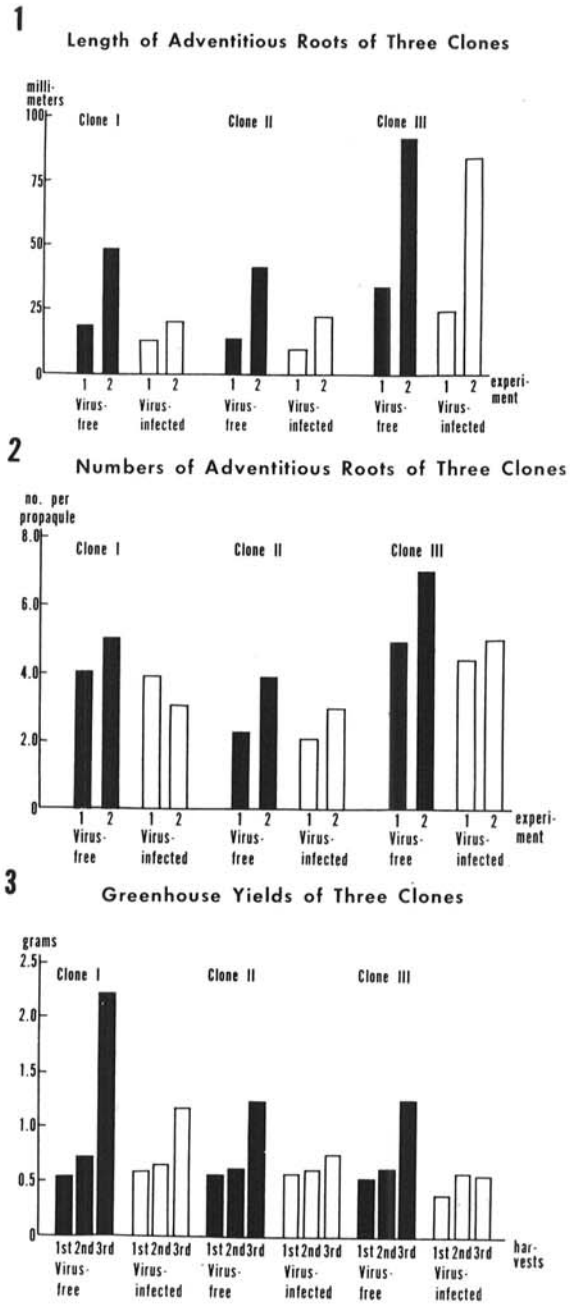

Fig. 1-3. Effects of tobacco ringspot virus on three clones of birdsfoot trefoil: greenhouse tests. **1)** Length of adventitious roots. **2)** Numbers of adventitious roots. **3)** Greenhouse yields.

54 plants; highest, 60 plants). Plants were later set in field plots with a 60-cm spacing within and between rows in a randomized complete block design with six entries and six replications. Each entry was represented in each replication at least 8 times. Unequal numbers of plants in subclasses were considered in the analysis of variance of plant vigor. Persistence data were recorded for each entry as the per cent living plants per replication at the end of the study in the fall of 1967.

At time of first flower in 1967, two vigorous plants of each entry/replication were selected for flower and seed production studies. We tagged a max of 50 umbels/plant as flowers opened, noting date and number of florets. Umbels were harvested before pods

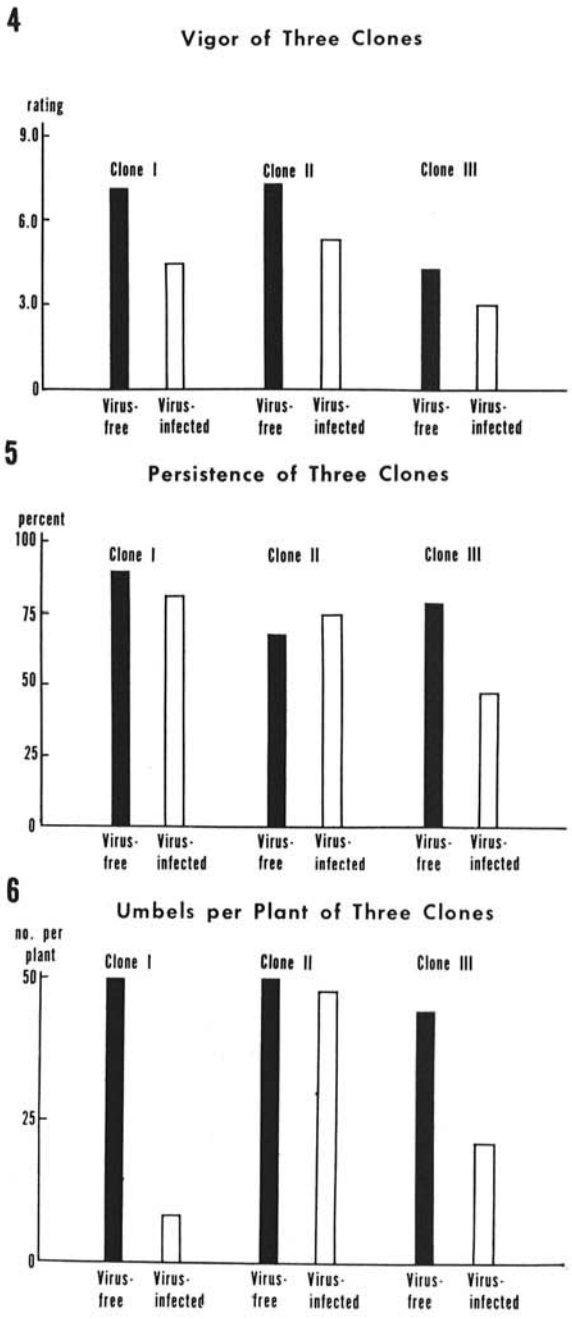

Fig. 4-6. Effects of tobacco ringspot virus on three clones of birdsfoot trefoil: field tests. **4)** Vigor. **5)** Persistence. **6)** Umbels per plant.

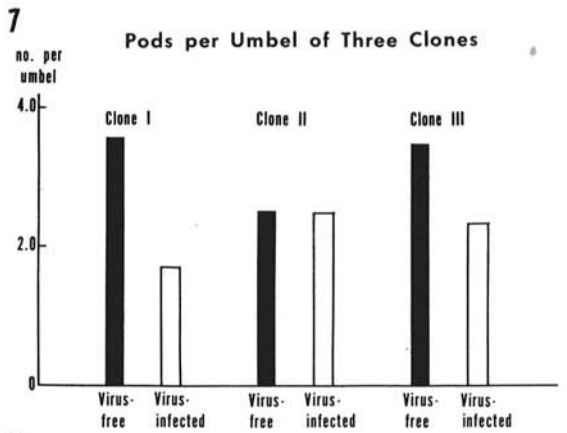

Fig. 7-9. Effects of tobacco ringspot virus on three clones of birdsfoot trefoil: field tests. **7)** Pods per umbel. **8)** Seeds per pod. **9)** Wt per 100 seed.

were completely dry to reduce seed shattering, then stored individually in small coin envelopes. Threshing, cleaning, counting, and final weighing of seed were done after moisture equilibrium was attained under laboratory storage conditions.

Results of field experiments are summarized in Fig. 4-9. In all tests, there was a highly significant difference between virus-free and virus-infected groups, and there were also significant clonal differences. Clone × virus interaction was significant for all characters except wt per 100 seed. Significance in clone × virus interaction was due, in part, to performance of clone II, which is apparently "tolerant" of TRSV infection as reflected by the measurements on persistence, umbels per plant, pods per umbel, and wt per 100 seed. In contrast, flowering in clone I was severely affected. Umbels on virus-infected clone I plants were few in number and developed much later than in their healthy counterparts. Numbers of pods per umbel and seed per pod on infected plants were markedly reduced.

DISCUSSION.—It is conceivable that a plant of average vigor, but possessing a desirable agronomic trait, such as profuse flowering habit (example: TRSV-infected clone II), could be inadvertently maintained in a selection program. Seed transmission of TRSV is common in soybean (1), and is known to be transmitted by certain nematode species (2). Other viruses are transmitted through pollen as well as through the ovule (10). The level of seed transmission of TRSV in birdsfoot trefoil has not yet been determined. In view of these known or suspected vectors, however, the presence of symptomless virus reservoirs in breeding plots of birdsfoot trefoil may be a threat to successful production.

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