



Forage Research in Texas

1983

Identification of Viruses Infecting Annual Clovers

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SUMMARY

Twenty - six annual clovers, including arrowleaf (Trifolium vesiculosum Savi.), subterranean (Trifolium subterraneum L.), white (Trifolium repens L.) and Trifolium mutabile were indexed for virus infection by enzyme-linked immunosorbent assay (ELISA). Red clover vein mosaic virus (RCVMV) and bean yellow mosaic virus (BYMV) were found in arrowleaf clover. BYMV was also found in subterranean clover and T. mutabile.

Introduction

Virus diseases are known to affect forage production of many legumes including arrowleaf clover (Gibson et al., 1979, Plant Disease 63:297-300). Infection by BYMV increased the severity of Phytophthora root rot on arrowleaf clover in Mississippi (Pratt et al., 1982, Phytopathology 72:1189-1192). Symptoms resembling those caused by virus infection (stunted plants, yellowing, crinkled and mottled leaves) were noted on arrowleaf plants in selection nurseries at Overton in 1981. Plans were made to sample arrowleaf and other clovers at Overton in 1982 in order to identify the virus(es) involved.

Procedure

Ten to twelve leaves were collected in May 1982 from each of 18 arrowleaf clover plants, 6 subterranean clover plants, one white clover plant and one T. mutabile plant. The double antibody sandwich ELISA was used to index the clover samples for virus infections. ELISA plates sensitized with specific antibodies to alfalfa mosaic virus (AMV), BYMV, clover yellow vein virus (CYVV), peanut stunt virus (PSV), RCVMV, and white clover mosaic virus (WCMV) were mailed (McLaughlin et al., 1979, Phytopathology 69:530) from Mississippi State, MS to Overton where leaf samples were prepared for testing. Leaf samples were crushed in 3.5 ml of phosphate buffered saline containing Tween 20 and NaDIECA (McLaughlin and Barnett, 1979, Phytopathology 69:1038). The resulting sap was transferred to test wells in the ELISA plates and incubated overnight in the refrigerator. The next morning the ELISA plates were rinsed with tap water to remove all traces of plant sap. The plates were then mailed to Mississippi State for completion of the assay and recording of results.

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Results and Discussion

Eleven arrowleaf clover plants with virus-like disease symptoms were sampled. RCVMV was identified in one plant, BYMV in six plants and none of the six viruses were detected in four plants. BYMV was detected in four of seven arrowleaf clover plants sampled that showed no virus-like disease symptoms. BYMV was identified from the T. mutabile plant sampled. BYMV was detected in one of three subterranean clover plants showing virus-like disease symptoms. None of the six viruses were detected in the white clover plant sampled nor in the three subterranean clover plants showing no symptoms. Results are summarized in Table 1.

This initial survey indicates the presence of virus diseases in annual clovers at Overton. Further research with virus diseases of annual clovers will be conducted in conjunction with Southern Regional Research Project S-127, Forage Legume Viruses. Objectives include identification of viruses affecting annual clovers and breeding for resistance to virus diseases.

Table 1. Clover Virus Investigation, Overton, Texas, May 24, 1982.

Species	Symptomatic	No. Plants	Viruses Detected (No. Infected Plants)
Arrowleaf	0 ¹	7	BYMV(4) ²
	+	2	BYMV(1)
	++	7	BYMV(3),RCVMV(1)
	+++	2	BYMV(2)
Subterranean	0	3	None
	++	3	BYMV(1)
White	0	1	None
<u>Trifolium mutabile</u>	++	1	BYMV(1)

¹0 = no symptoms, + = mild symptoms, ++ = intermediate symptoms, +++ = severe symptoms.

²Plants were indexed by ELISA for alfalfa mosaic virus, bean yellow mosaic virus (BYMV), clover yellow vein virus, peanut stunt virus, red clover vein mosaic virus (RCVMV) and white clover mosaic virus.