EFFECTS OF TWO SOIL-BORNE VIRUSES OF SUGARBEET AND THEIR FUNGAL VECTOR, *POLYMYXA BETAE*, ON VIRUS ACCUMULATION AND PLANT GROWTH IN SUGARBEET.

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ABSTRACT

Soils naturally infested with cultures of aviruliferous *Polymyxa betae* and viruliferous *P. betae* carrying the two sugar beet benyviruses *Beet necrotic yellow vein virus* (BNYVV) and *Beet soil-borne mosaic virus* (BSBMV), alone and in combination, were compared to non-infested soil with regard to their effects on virus content, fresh plant weight, and seedling emergence. Two sugar beet varieties were used: a diploid (*Rzrz*) that carries resistance to rhizomania caused by BNYVV, and a triploid rhizomania-susceptible variety (*rzrzrz*). These studies clearly demonstrated that the Rz resistance gene does not confer resistance to BSBMV. Additionally, *P. betae* alone had a significant negative effect on growth of sugarbeet, and soils infested with *P. betae* containing one or both viruses, tended to have reduced seedling emergence and reduced fresh weight, even when protective fungicides were used. BSBMV titers were significantly higher in single infections than in mixed infections with BNYVV in both rhizomania resistant and susceptible varieties. In contrast, BNYVV titers were very high in single and in mixed infections in the Rhizomania-susceptible variety, but low in the resistant variety. Therefore, in the absence of BNYVV, BSBMV concentrations are high in infected roots, regardless of the resistance genotype. In the presence of BNYVV, however, BSBMV concentrations are low in both resistant and susceptible varieties, with absorbance readings similar to those of plants grown in non-infested soils. It appears that even at low levels, BNYVV either out competes or suppresses BSBMV, and suggests that both viruses target similar cellular processes in the sugarbeet plant.

INTRODUCTION

Beet necrotic yellow vein virus (BNYVV) and Beet soil-borne mosaic virus (BSBMV) are members of the genus Benyvirus in the Furoviridae (13,15). Both viruses infect members of the Chenopodiaceae, most notably sugarbeet (Beta vulgaris L.), and both are transmitted by Polymyxa betae (1,2). BNYVV has been shown to be the cause of rhizomania (4,12). BSBMV was first described in the United States by Duffus and Liu in 1987 (5), and has been shown to have a sequence similarity to BNYVV that ranged from 30-90% in RNA2 and a genome organization similar to BNYVV (7). Based on these and other studies (17), and the fact that BSBMV does not cause rhizomania, BSBMV is considered to be a distinct member of the genus Benyviridae.

Because of the international significance and economic losses caused by BNYVV on sugar beet production, a breeding program has been in place in the U.S. since 1984 (3,8,9,10). A gene that induces resistance to rhizomania in sugar beet (Rz) was identified in 1983 from sugar beet and subsequently from wild beet (B. maritima) (3,10). Breeding programs have improved rhizomania resistance to a point where, even under conditions of high inoculum (i.e., the San Joaquin Valley, California), yields and sugar production are equal to those of high-yielding, susceptible sugar beet varieties in the absence of rhizomania (Lewellen, unpublished data). Furthermore, the level of resistance is significantly correlated with the dose of the Rz allele as measured by (i) root weight, (ii) the rhizomania disease index (DI) rating (from 1-9, where a low DI indicates resistance and lack of symptoms, and a high DI indicates susceptibility and the presence of hairy roots, a wine glass-shaped root and internal necrosis), and (iii) sugar yield. For example, a strong negative correlation was shown between a decreasing dosage of the Rz allele and absorbance and the DI rating (Rzrz<Rzrzrz<rzrzrz). However, a positive correlation was shown between a decreasing dosage of the Rz allele and root weight (Rzrz>Rzrzrz>rzrzrz) (16). A diploid rhizomania-resistant variety (Rzrz) has a lower virus titer and DI rating but a higher root weight than a triploid resistant variety (Rzrzrz), and a susceptible triploid variety (rzrzrz). Homozygous resistance (RzRz) usually conditions a higher level of resistance than the heterozygous (Rzrz) genotype (Lewellen, unpublished data).

In contrast to BNYVV, little is known about the effect of BSBMV on yield and sugar production in sugar beet. The objectives of this study were: (i) to determine if the Rz gene confers resistance to BSBMV, and (ii) to determine the effects of BNYVV and BSBMV, alone and in combination, on the relative virus titers in sugar beet. Our goal in this study was to determine the effect of single and mixed infections of benyvirus in naturally infested soils.

MATERIALS AND METHODS

Methods to test soils for rhizomania have been described by Gerik et al. (6). Soil samples previously identified as being singly infested with BNYVV, BSBMV, or aviruliferous *P. betae* have been increased and stored at 4C for this study. BNYVV-infested soil was taken from the sugar beet fields that had been infested since the late 1980's and consistently used in rhizomania variety trials at the USDA-ARS in Salinas, CA. Tests are routinely made in these rhizomania fields for the presence of BSBMV, and it has never been detected. BSBMV-infested soil was obtained from sugar beet fields in Nebraska and was submitted by Dr. Eric Kerr (University of Nebraska). Aviruliferous, *P. betae*-infested soil was obtained from river sand provided by Dr. Gary D. Franc (University of Wyoming). To increase the quantities of infested soil samples, roots from pot cultures were air dried, homogenized in mortars and pestles, and thoroughly mixed into respective soil samples. Non-infested soil consisted of loamy sand collected from the nearby dry bed of the Salinas River, and that was autoclaved prior to use. A list of computer-generated random numbers was used to determine the placement of each pot on the greenhouse benches for a completely random design. Varieties used in tests 1, 2, and 3 were Beta4330R (*Rzrz*; resistant) and KWS6770 (*rzrzrz*; susceptible).

Test 1 consisted of the following treatments: (1) non-infested soil, (2) BSBMV-infested soil, (3) BNYVV-infested soil and (4) BNYVV- and BSBMV-infested soil, mixed in equal parts. In this test, only the rhizomania-susceptible variety (KWS6770) was used. Samples were harvested weekly for 6 weeks starting 2 weeks post emergence of seedlings. Each treatment combination (soil × harvest date) consisted of six pots each for a total of 24 pots weekly.

In tests 2 and 3, aviruliferous *P. betae* was added as a treatment, and a rhizomania-resistant variety (Beta 4430R) was also included. In these two tests, each treatment combination (soil × variety × harvest date) consisted of three pots each. Roots from these pots were harvested and tested at weekly intervals for 6 weeks, for a total of 30 pots weekly.

In previous studies (11,14) a clear relationship was obtained between virus concentrations in BNYVV-infected plants and absorbance values with ELISA. A triple antibody sandwich (TAS)-ELISA was developed in collaboration with Agdia, Inc. (Elkhart, IN) that was specific for BNYVV. The double antibody sandwich (DAS)-ELISA test was used to test for BSBMV. Antiserum to BSBMV was provided by H.-Y. Liu. Absorbance readings (A_{405nm}) from the average of paired wells were made with a Bio-Tek EL312e microplate reader (Winooski, VT). All reported ELISA values were for the 2 hr period and represent the ratio of the test sample absorbance at A_{405nm} divided by the absorbance of the healthy sample. Ratios of ≥3 times the healthy mean were considered to be positive. Data were obtained for each individual pot and used in statistical analyses

RESULTS

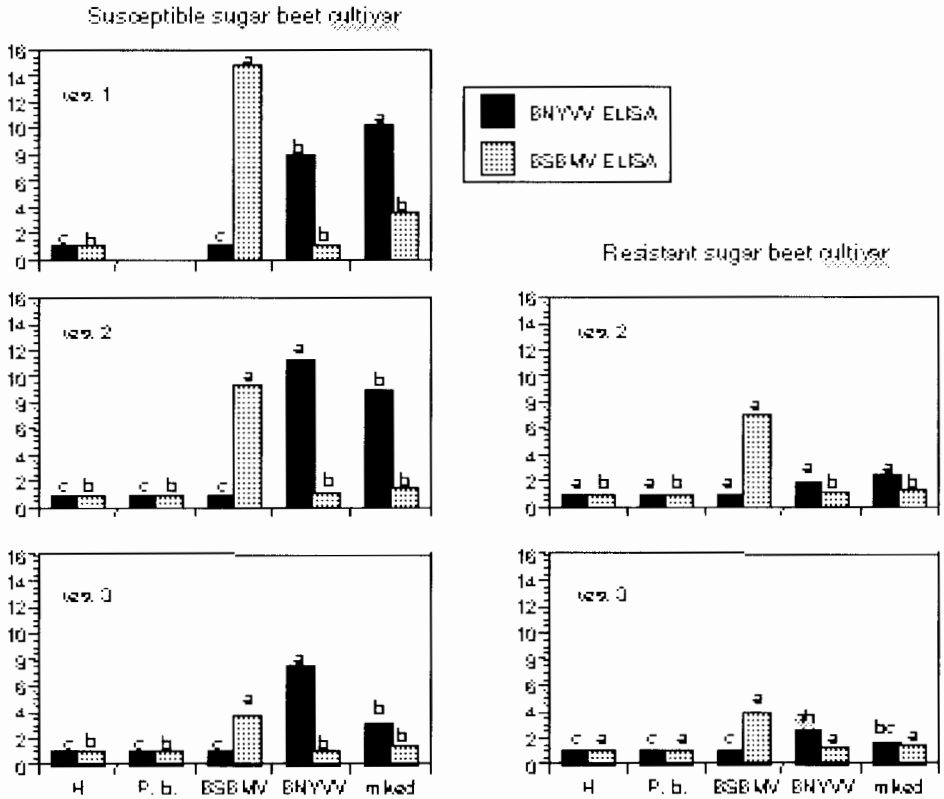
Resistance to rhizomania caused by BNYVV and conferred by the *Rz* allele did not confer resistance to BSBMV in sugar beet in greenhouse pot cultures (Fig. 1). Titers of BNYVV in the rhizomania susceptible variety (7-11 times the healthy mean) were significantly higher than in the resistant variety (<2 times the healthy mean). Slight differences occurred for the titers of BSBMV between these varieties when tested as single infections across all six harvest dates. A significant variety × soil treatment interaction occurred for BNYVV but not for BSBMV.

In test 1, where only the rhizomania-susceptible variety was used, titers of BSBMV were significantly reduced from strongly positive as single infections (almost 15 times the healthy mean), to values only 3.5 times higher than healthy mean values when in mixed infections with BNYVV. In contrast, titers of BNYVV were 7.9 times the healthy mean in single infections and 10.2 times in mixed infections. In tests 2 and 3, titers of BSBMV were significantly reduced in mixed infection soil compared to single infections with either rhizomania-susceptible or resistant varieties (Figure 1). Titers of BNYVV were positive in the susceptible variety. In test 3, the titers of BNYVV are decreased from 7.5 to 3.1 times the healthy mean between single and mixed infections, but readings were still considered positive in both cases. For the resistant variety, BNYVV ELISA ratios were consistently in the negative range (1.7 to 2.5 times the healthy mean), regardless of whether they existed as single or as mixed infections.

DISCUSSION AND CONCLUSIONS

Several conclusions were made for the effects of (i) BSBMV, and (ii) mixed infections of BNYVV and BSBMV on sugar beet in greenhouse pot culture. The three tests reported here were conducted sequentially over one year, with different day-lengths and growth potential throughout the year. Nevertheless, the same significant effects were observed.

Fig. 1. Absorbance values (A_{405nm}) of test samples divided by the healthy means for susceptible (rzrzrz) and resistant (Rzrz) sugar beet cultivars. Test 1 consists of four soil treatments and tests 2 and 3 consist of five soil treatments. Bars with letters in common within ELISA comparisons for each individual virus are not significantly different within a test at the p=0.05 level.



BSBMV alone replicates to high levels in both resistant and susceptible rhizomania varieties. However, when BSBMV exists as mixed infections with BNYVV, the levels of BSBMV are significantly reduced, even when BNYVV levels are extremely low, as seen in the rhizomania resistant variety. Titers of BSBMV were significantly reduced in the rhizomania resistant variety as well even with extremely low titers of BNYVV. The titers of BNYVV were also reduced in tests 2 and 3 in the susceptible variety, when in combination with BSBMV, but the titers of BNYVV observed are still considered positive. The significant reduction of BSBMV when in combination with BNYVV could be due

to several factors. There may be competition for infection sites by viruliferous *P. betae*. Alternatively, BNVYY-infected zoospores of *P. betae* may be more aggressive than BSBMV-infected *P. betae*. The viruses may have a competitive advantage once inside the host cells. Another possible explanation for the relationship between these two viruses in mixed infections involves competition for replicative or movement proteins inside the host cells. Regardless of how these two viruses interact in sugar beet, attention should be paid to the negative effect that BSBMV has on beet production. Efforts should be made to determine the extent of the effect of this virus on field production, and to identify sources of resistance to BSBMV.

ACKNOWLEDGEMENTS

This research has been supported in part by grants from the Beet Sugar Development Foundation, California Beet Growers' Association, Western Sugar Research and Development Foundation.

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