Evaluation of Dichloropropene Fumigant and Benzothiadiazole Seed Treatment on Sugarbeet in a Rhizomania Infested Field

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ABSTRACT

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV) and transmitted by the plasmodiophorid *Polymyxa betae*, is an emerging disease that is spreading quickly throughout the sugarbeet (*Beta vulgaris*) production region of the Red River Valley in North Dakota and Minnesota. Although productive resistant sugarbeet cultivars are available, alternative methods of management need to be identified in case resistance genes become ineffective. A study was conducted near Glyndon, MN in 2003 and 2004 to evaluate dichloropropene soil fumigant and benzothiadiazole systemic acquired resistance inducer as a seed treatment on performance of sugarbeet cultivars differing in susceptibility to BNYVV in a field infested with rhizomania. In 2003, the BNYVV resistant cultivar consistently outperformed the susceptible cultivar; however, no differences among dichloropropene fumigated plots, plots planted with benzothiadiazole treated seed, and untreated control plots were detected. In 2004, no differences among cultivars were observed for any measured variables, and untreated control plots generally outperformed plots fumigated with dichloropropene. From this research, dichloropropene fumigation and benzothiadiazole as a seed treatment are not suitable rhizomania management practices for the Red River Valley sugarbeet production region of North Dakota and Minnesota.

Additional key words: Actigard, *Beet necrotic yellow vein virus*, *Beta*

vulgaris, Bion, *Polymyxa betae*, Telone

Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV), has spread throughout the Red River Valley of North Dakota and Minnesota. BNYVV is transmitted by the soilborne plasmodiophorid *Polymyxa betae* Keskin. Rhizomania has been managed in North Dakota and Minnesota with the use of resistant cultivars that contain the *Rz1* gene and long crop rotations. Strains of BNYVV have recently been found in Europe (Harju et al., 2002) and California (Liu et al., 2005) that are able to overcome the *Rz1* gene for resistance. The appearance of rhizomania symptoms in sugarbeet (*Beta vulgaris* L.) fields planted to resistant cultivars in the Red River Valley and southern Minnesota sugarbeet production areas also may indicate the presence of new strains of BNYVV that are able to overcome the *Rz1* gene. Due to these concerns, additional methods of controlling rhizomania may be needed until more durable resistant genes are identified and incorporated into commercial cultivars. The use of fumigation has been evaluated for control of rhizomania in California, Texas, and the United Kingdom (Harveson and Rush, 1994; Henry et al., 1992; Martin and Whitney, 1990). The systemic acquired resistance (SAR) inducer benzothiadiazole manufactured by Syngenta Crop Protection (Greensboro, NC), and marketed as Actigard in the United States and Bion in Europe, has been evaluated for control of rhizomania in Germany (Mouhanna and Schlosser, 1998). Neither soil fumigation nor benzothiadiazole has been evaluated for rhizomania management in the Red River Valley of the northern United States. The objective of this study was to evaluate the effect of fumigation with dichloropropene and seed treatment with benzothiadiazole on rhizomania susceptible and resistant sugarbeet cultivars in a rhizomania infested field located in the Red River Valley of Minnesota.

MATERIALS AND METHODS

A rhizomania resistant sugarbeet cultivar (VDH 46177) and a susceptible cultivar (ACH 999 in 2003; ACH 952 in 2004) were planted near Glyndon, MN on 23 May and 27 April in 2003 and 2004, respectively. The research site was located on a commercially-farmed field that had a history of rhizomania; different locations within the site were used each year. Two chemical treatments were evaluated and compared to an untreated control for each cultivar. The chemical treatments consisted of plots fumigated with dichloropropene (Telone II, DowAgroSciences, Indianapolis, IN) and plots planted with seed treated with benzothiadiazole (Actigard 50 WG, Syngenta Crop Protection,

Greensboro, NC) at 1.5 g a.i./kg seed. The dichloropropene was applied into the soil with chisels spaced 56 cm apart at a 30 cm depth at 132 kg a.i./ha (112 L/ha product) on 6 November and 9 October in 2002 and 2003, respectively; soil was immediately packed with a roller after the dichloropropene application. Plots were grown using standard agronomic practices, which included herbicide applications for weed control and foliar fungicide applications for Cercospora leaf spot (caused by *Cercospora beticola* Sacc.) control. The soil type at the field location was a Glyndon loam (coarse silty, mixed, superactive, frigid, aeric calciaquoll; 3% organic matter). A minimum of 5 roots per plot were collected for BNYVV testing. Lateral roots from each beet were removed and washed with water to remove adhering soil. Approximately 0.5 g of the lateral root tissue was homogenized in 2 ml of phosphate-buffered saline containing 0.05% Tween 20 at pH 7.4. The presence of BNYVV was analyzed using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) technique with a BNYVV reagent set (Agdia, Elkhart, IN). Absorbance values of each DAS ELISA reaction were obtained using an ELISA plate reader (Titertek Multiscan, Titertek Instruments, Huntsville, AL) at 405 nm. Roots were harvested on 1 October and 7 October in 2003 and 2004, respectively. Sugar concentration and loss to molasses was determined at the American Crystal Sugar Company Quality Laboratory (East Grand Forks, MN). Plots were 4 rows wide on 56 cm centers, 9 m long, and arranged as a randomized complete block design with 4 replications; adjacent plots were separated by two untreated buffer rows. The general linear model procedure (PROC GLM) in SAS (SAS Institute, Inc., Cary, NC) was used for statistical analysis. Fisher's protected least significant difference (LSD), where $\alpha = 0.05$, was used to compare treatment means.

RESULTS

Because different susceptible cultivars were used in each year, years were analyzed separately. No significant ($P \leq 0.05$) cultivar x treatment interactions were detected in either year; therefore, main effects only are presented.

In 2003, the rhizomania resistant cultivar VDH 46177 had significantly greater sucrose concentration, sucrose yield, and root yield than ACH 952 (Table 1). No significant difference between cultivars for ELISA reaction was observed in 2003. No significant differences between the cultivars were observed for any of the measured variables in 2004 (Table 2).

In 2003, no significant differences among the untreated control,

Table 1. Comparison of a rhizomania resistant and susceptible sugarbeet cultivar at Glyndon, MN in 2003.

Cultivar	BNYVV*	Sucrose concentration (%)	Recoverable sucrose yield (kg/ha)	Root yield (Mg/ha)
VDH 46177 (Res.)	0.024	16.2	4841	32
ACH 999 (Susc.)	0.031	14.6	3044	22
$P > F$	0.392	0.001	0.007	0.032

*Absorbance readings ($A_{405\text{nm}}$) of *Beet necrotic yellow vein virus* in sugarbeet

Table 2. Comparison of a rhizomania resistant and susceptible sugarbeet cultivar at Glyndon, MN in 2004.

Cultivar	BNYVV*	Sucrose concentration (%)	Recoverable sucrose yield (kg/ha)	Root yield (Mg/ha)
VDH 46177 (Res.)	0.112	16.5	6168	40
ACH 952 (Susc.)	0.531	16.3	6852	44
$P > F$	0.072	0.097	0.177	0.105

*Absorbance readings ($A_{405\text{nm}}$) of *Beet necrotic yellow vein virus* in sugarbeet

dichloropropene fumigation, and benzothiadiazole treated seed were observed for any of the measured variables (Table 3). In 2004, no significant differences among these treatments for ELISA reaction or sucrose yield were observed; however, significant differences did occur among treatments for sucrose concentration and root yield (Table 4). Roots harvested from untreated control plots had significantly greater sucrose concentration than plots fumigated with dichloropropene in 2004. Sucrose concentration of plots planted to seed treated with benzothiadiazole did not significantly differ between untreated or dichloropropene fumigated plots. Untreated plots had significantly greater root yield than dichloropropene fumigated plots, but were not significantly different than plots planted with benzothiadiazole-treated seed in 2004.

Table 3. Comparison of rhizomania management treatments on sugarbeet at Glyndon, MN in 2003.

Treatment	BNYVV*	Sucrose concentration (%)	Recoverable sucrose yield (kg/ha)	Root yield (Mg/ha)
Untreated	0.026	15.5	4198	30
Benzothiadiazole	0.031	15.4	3642	25
Dichloropropene	0.025	15.3	4102	27
LSD 0.05	NS	NS	NS	NS

*Absorbance readings (A_{405nm}) of *Beet necrotic yellow vein virus* in sugarbeet roots after double-antibody sandwich enzyme-linked immunosorbent assay.

Table 4. Comparison of rhizomania management treatments on sugarbeet at Glyndon, MN in 2004.

Treatment	BNYVV*	Sucrose concentration (%)	Recoverable sucrose yield (kg/ha)	Root yield (Mg/ha)
Untreated	0.592	16.7	7176	47
Benzothiadiazole	0.330	16.4	6674	42
Dichloropropene	0.043	16.1	5678	37
LSD 0.05	NS	0.4	NS	7

*Absorbance readings (A_{405nm}) of *Beet necrotic yellow vein virus* in sugarbeet roots after double-antibody sandwich enzyme-linked immunosorbent assay.

DISCUSSION

No benefits of soil fumigation with dichloropropene were observed in our research trials. This is in contrast to results reported by Harveson and Rush (1994) or Martin and Whitney (1990), in which a benefit with dichloropropene soil fumigation was observed in Texas and California, respectively. The dichloropropene applied in this study and the Texas (Harveson and Rush, 1994) and the California (Martin and Whitney, 1990) studies was formulated as Telone II. Telone II was applied at 112 L/ha in our study, which was a greater use rate than that used in the Texas (93 L/ha) or the California (68 L/ha) study. Because our study used a higher rate of Telone II, the likelihood of observing a benefit was greater; however, no benefits with Telone II were observed in our research. Differences in cultivars used, level of organic material in the

soils, and soil temperatures at application for the different studies possibly could account for the different results.

The study by Martin and Whitney (1990) was conducted using only one susceptible cultivar, and the study by Harveson and Rush (1994) was conducted using several cultivars with differing levels of resistance. Our study evaluated only two cultivars each year. Harveson and Rush (1994) reported that a benefit with the use of dichloropropene occurred on some cultivars but not others. It is possible that other cultivars not evaluated in our trials could have responded differently with fumigation.

Organic matter content of soil can play a role in the effectiveness of dichloropropene. As organic matter increases in the soil, the effectiveness of dichloropropene as a pesticide may be reduced (Gan et al., 1998; Guo et al., 2004; Thomas et al., 2004). The soil at the Glyndon, MN site contained 3% organic matter, and typical soils in the Red River Valley of North Dakota and Minnesota have a high (2.5% – 6.5%) amount of organic matter (D. Franzen, personal communication). The level of organic matter in the soil at the Texas trials conducted by Harveson and Rush (1994) was 1.6%; soil organic matter was not reported in the California trials conducted by Martin and Whitney (1990).

Dichloropropene was applied to our study in autumn, when soil temperatures were low (4ºC and 19ºC in 2003 and 2004, respectively), and plots were planted in the following spring. The use label for Telone II states that soil temperatures should be between 5ºC and 27ºC when applied. It is possible that the low soil temperature during dichloropropene application may have inhibited its effects on the *P. betae*, especially for the plots in the 2003 growing season. Harveson and Rush (1994) applied dichloropropene in February and planted their plots in April. The differences in intervals between fumigation and planting could have lead to contrasting results. With the longer interval in our studies, there would be a greater chance of contaminated soil moving into the fumigated plot areas.

Benzothiadiazole, applied to sugarbeet leaves, has been shown to induce β-1,3-glucanase and chitinase isozymes, which are pathogenesis-related (PR) proteins (Burketova et al., 1999). Sugarbeet roots inoculated with BNYVV were shown to induce the accumulation of β-1,3-glucanase and chitinase, which were also induced by benzothiadiazole (Burketova et al., 2003). Because of these results, Burketova et al. (2003) suggested that benzothiadiazole had the potential to protect sugarbeet roots against BNYVV. Mouhanna and Schlosser (1998) reported that benzothiadiazole applied as a seed treatment at 0.5 g a.i./kg seed reduced BNYVV titer compared to an untreated control in a BNYVV tolerant cultivar, but not in a susceptible cultivar. Because the Mouhanna and Schlosser (1998)

study was conducted in the greenhouse and plants were evaluated for BNYVV after only 7 weeks of growth, the rate of benzothiadiazole used in our study was much greater to determine if season-long protection was possible (1.5 g a.i./kg seed). Even though a high rate of benzothiadiazole was used in our study, no benefit from the use of benzothiadiazole was observed. It is possible that roots from benzothiadiazole-treated seed may have had lower BNYVV titer early in the season, as we only measured for BNYVV once, which was later in the season. If this was the case, then any protection against BNYVV due to the benzothiadiazole treatment did not last later into the growing season.

From this research, dichloropropene fumigation and benzothiadiazole as a seed treatment apparently are not suitable rhizomania management practices for the Red River Valley of North Dakota and Minnesota; however, different cultivars may have reacted differently to these treatments. Management of rhizomania in this region should continue to utilize crop rotation and resistant cultivars. Because of the threat of new strains of BNYVV developing in this region that can overcome the *Rz1* gene for resistance, it is important that plant pathologists and breeders continue to screen germ plasm for new effective and durable sources of resistance.

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