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Effect of Soil Temperature, Injection Depth, and Metam Sodium Rate on the Management of Verticillium Wilt of Potato

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Abstract Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a primary component of the early dying complex of potato (*Solanum tuberosum* L.) in the United States. Although genetic resistance to *V. dahliae* exists and has been incorporated into several potato cultivars, the commercial potato industry is still dominated by cultivars susceptible to the pathogen. As a result, soil fumigation with metam sodium remains an important means by which Verticillium wilt is controlled, despite its expense and potentially negative environmental impact. Recent restrictions on metam sodium use by the Environmental Protection Agency directed at reducing exposure to vapor emissions have increased the need to improve shank injection of the soil fumigant. In studies reported here, the application of metam sodium reduced the severity of Verticillium wilt, however, soil temperature at the time of injection, metam sodium injection depth, and application rate had little overall effect. In 2011, temperature at the time of metam sodium injection did not result in significant differences in any parameter evaluated. However, in 2012, soil populations of *V. dahliae*, wilt severity and host colonization were significantly reduced when metam sodium was applied at 4 °C compared to 13 or 15 °C. No significant differences were observed between a single or two metam sodium injection depths in any parameter evaluated across the 2 years the study was conducted. While all rates of metam sodium significantly reduced soil populations of *V. dahliae* compared to the non-treated control, significant differences across rates were rarely observed. Improved control of Verticillium wilt and increased yield can be achieved as a result of these studies. The effective control of Verticillium wilt can be obtained by using metam sodium at a comparatively low rate of 373 l/ha, particularly when applied at a relatively cold soil temperature of 4 °C using a single injection depth of 25 cm. The potential impact of these application modifications of metam sodium in reducing emissions and non-target exposure is discussed.

Resumen La marchitez por Verticillium, causada por *Verticillium dahliae* Kleb., es un componente primario del complejo de muerte temprana de papa (*Solanum tuberosum* L.) en los Estados Unidos. Aun cuando existe resistencia genética a *V. dahliae* y se ha incorporado a varias variedades de papa, la industria de la papa comercial aún está dominada por variedades susceptibles al patógeno. Como resultado, la fumigación del suelo con metam sodio permanece como un medio importante por el cual se controla la marchitez por Verticillium, a pesar de que es caro y de su impacto ambiental potencialmente negativo. Restricciones recientes en el uso de metam sodio por la Agencia de Protección al Ambiente dirigidas a la reducción de la exposición a las emisiones de vapor, han aumentado la necesidad de mejorar las inyecciones del fumigante del suelo. En estudios aquí reportados, la aplicación del metam sodio redujo la severidad de la marchitez por Verticillium, no obstante, la temperatura del suelo al momento de la inyección, la profundidad de ésta, y la dosis de aplicación, tuvieron poco efecto en general. En 2011, la temperatura al momento de la inyección del metam sodio no resultó en diferencias significativas en cualquier parámetro. No obstante, en 2012, las poblaciones del suelo de *V. dahliae*, la severidad del marchitamiento y la colonización del hospedante se redujeron significativamente cuando el metam sodio se aplicó a 4 °C comparado a 13º o 15 °C. No se observaron diferencias significativas entre una o

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dos profundidades de inyección de metam sodio en cualquier parámetro evaluado a lo largo de los dos años de la conducción del estudio. Mientras todas las dosis de metam sodio redujeron significativamente las poblaciones en el suelo de *V. dahliae* comparadas con el testigo sin tratar, raramente se observaron diferencias significativas entre los niveles. El mejoramiento en el control del marchitamiento por Verticillium y el incremento en el rendimiento se pueden lograr como resultado de estos estudios. El control efectivo de la marchitez por Verticillium se puede obtener mediante el uso de metam sodio a una dosis comparativamente baja de 373 l/ha, particularmente cuando se aplica a una temperatura relativamente fría del suelo de 4 °C usando una sola profundidad de inyección de 25 cm. Se discute el impacto potencial de estas modificaciones de aplicación de metam sodio en la reducción de emisiones y de exposición a no-objetivos.

Keywords *Verticillium dahliae* · Early dying · Soil fumigation · Shank injection · *Solanum tuberosum*

Introduction

Verticillium wilt of potato (*Solanum tuberosum* L.) is caused primarily by *Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke and Berthold. *V. dahliae* is the most widespread and destructive of the two pathogens primarily because microsclerotia persist in the soil for as many as 14 years (Powelson and Rowe 1993; Wilhelm 1955). This pathogen generally is regarded as the most important component of the potato early dying complex in the United States (Davis 1985; Martin et al. 1982; Powelson and Rowe 1993; Rowe 1985; Rowe et al. 1987; Rowe and Powelson 2002; Rowe et al. 1985). Due to the ease of introduction of *V. dahliae* into non-infested fields through the vegetative propagule, the tuber, (Dung and Johnson 2012), a wide host range, and the longevity of microsclerotial survival in the soil, most agricultural soils are infested with the pathogen to some extent (Rowe 1985; Powelson et al. 1993). Effects on potato yield are most serious when the crop is grown in short or continuous potato rotations (Davis et al. 1994) and in the presence of high soil populations of the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Steckh. (Burpee and Bloom 1978; Martin et al. 1982; Rowe et al. 1985). Additionally, soil populations of *V. dahliae* can build-up and survive for long periods in the presence of conservation tillage cropping practices (Bockus and Shroyer 1998; Taylor et al. 2005).

The symptoms of successful infection by *V. dahliae* include wilting of the host plant, premature vine death, and chlorosis and necrosis of foliage (Davis and Huisman 2001; Rich 1983). Infected vascular bundles appear light to dark brown and plants remain erect after senescence, a symptom unique to Verticillium wilt that distinguishes it from symptoms of other wilt diseases of potato (Rich 1983; Rowe 1985). However, symptoms of Verticillium wilt may not be evident until plants reach maturity and may be difficult to distinguish from natural senescence (Davis 1985).

Soil inoculum densities of *V. dahliae* have been shown to be correlated with the incidence and severity of Verticillium wilt (Ben-Yephet and Szmulewich 1985; Davis and Everson 1986; Nicot and Rouse 1987a, b; Nnodu and Harrison 1979; Taylor et al. 2005), however, not in every field (Davis and Everson 1986; Taylor et al. 2005). The lack of relationship between soil inoculum density and wilt severity has been found to be due to edaphic factors such as sodium content in soil, level of soil organic matter, and native soil fertility such as organic nitrogen availability (Davis and Everson 1986; Davis et al. 2001; Taylor et al. 2005). Physical properties including soil water content, aeration, compaction, texture and temperature, among others also can affect the development of soil borne diseases (Rothrock 1992). The use of synthetic fertilizers, as well as changes in agricultural cultural practices including tillage and irrigation, have been shown to impact Verticillium wilt development over the past several decades (Cappeart et al. 1992; Davis and Everson 1986; Taylor et al. 2005). The use of good cultural practices can alter these soil conditions, and an effective integrated crop disease management scheme must take into consideration all of these factors in order to positively influence the management of soil borne diseases such as Verticillium wilt.

Verticillium wilt management strategies and tactics are generally typical for a soil borne disease. Crop rotation, soil solarization, green manures, timing of planting and harvest, destruction of crop residue, irrigation scheduling, and soil fertility all have been studied and employed to manage this disease (Cappeart et al. 1992; Davis 1985; Davis and Huisman 2001; Davis and Sorenson 1986; Davis et al. 1996; Larkin et al. 2011; Powelson et al. 1993; Powelson and Rowe 1993; Rich 1983; Rowe et al. 1987; Rowe and Powelson 2002). Genetic resistance also is an effective management strategy, particularly in the French fry processing industry where genetic resistance was identified nearly 30 years ago and subsequently incorporated into a number of potato cultivars (Corsini et al. 1985, 1990; Jansky 2009; Pasche et al. 2013b). It has been long recognized that an important component for assessing true resistance to *V. dahliae* in breeding clones is to quantify vascular colonization or fungal biomass of the pathogen (Davis et al. 1983b; Frost, et al. 2007; Hoyos et al. 1991; Hu et al. 1993; Pasche et al. 2013a). Vascular colonization of *V. dahliae* in potato stems is also an important component of Verticillium wilt disease management since highly susceptible potato cultivars return large populations of the pathogen to the soil. Additionally, these propagules are difficult to manage because of protection provided by plant debris and, therefore, they may re-enter the soil gradually over multiple seasons as the debris decomposes

(Bae et al. 2007; Hoyos et al. 1991; Pasche et al. 2013b; Taylor et al. 2005). Unfortunately, Verticillium wilt resistant cultivars are not widely grown, largely due to a lack of consumer acceptance, particularly in the French fry processing industry (Pasche et al. 2013b). Soil fumigation using either metam sodium (Ben-Yephet et al. 1983; Davis et al. 1983a; Hamm et al. 2003; Powelson and Rowe 1993; Rowe et al. 1987; Taylor et al. 2005; Tsror et al. 2005) or biofumigants (Larkin and Griffin 2007; Larkin et al. 2011; McGuire 2003) frequently are used to manage Verticillium wilt in potato. Despite its expense and potentially negative environmental impact, metam sodium remains one of the most commonly used disease management tactics for Verticillium wilt in potato (Cox 2006; MacRae and Noling 2010; Rowe and Powelson 2002; Taylor et al. 2005).

Metam sodium has been used extensively to control soil borne pathogens of a number of crop plants and has been shown to effectively manage Verticillium wilt of potato (Ben-Yephet et al. 1983; Davis 1985; Davis et al. 1983a; Larkin et al. 2011; Powelson and Rowe 1993; Rowe and Powelson 2002; Taylor et al. 2005; Tsror et al. 2005) and other soil borne potato pathogens (Collins et al. 2006; Hamm et al. 2003). The principal breakdown product of metam sodium in soil is methyl isothiocyanate (MITC), a highly toxic biocide with significant potential for volatilization (Saeed et al. 2000; Smelt and Leistra 1974; Sullivan et al. 2004; Zhang and Wang 2007; Zheng et al. 2006). However, the efficacy of metam sodium in the control of Verticillium wilt of potato can be variable and has been associated with method of application (Ben-Yephet et al. 1983; Taylor et al. 2005). The application of metam sodium through the irrigation sprinkler system has proven to be reliably efficacious (Ben-Yephet et al. 1983; Taylor et al. 2005). Unfortunately, recent Environmental Protection Agency (EPA) regulations regarding metam sodium application makes sprinkler irrigation applications very difficult to perform due to increased regulatory restrictions and the need for extensive buffer zones to protect humans and wildlife (MacRae and Noling 2010). Therefore, shank injection of metam sodium is becoming more popular because of reduced vapor emissions (Saeed et al. 2000; Zheng et al. 2006). As a result, there is a need to more fully understand shank injection of metam sodium into the soil to improve Verticillium wilt control, while also potentially reducing MITC emissions and non-target impacts (Macalady et al. 1998; Sullivan et al. 2004; Yates et al. 2002; Zhang and Wang 2007; Zheng et al. 2006).

A number of factors including soil temperature at the time of application (Ben-Yephet and Frank 1985; Leistra and Smelt 1974; Saeed et al. 1997; Smelt and Leistra 1974); soil texture or soil particle size (Ben-Yephet and Frank 1985), soil moisture content (Saeed et al. 1997), metam sodium rate (Klose et al. 2008; Tsror et al. 2005), method of application (Taylor et al. 2005), organic matter content of the soil (Ben-Yephet and Frank 1985), and tillage (Taylor et al. 2005) have been shown to influence the efficacy of metam sodium. Although a number of studies have examined one or two factors on the efficacy of metam sodium, no study has attempted to investigate a three-way interaction of important application variables. The primary objective of the research studies reported here are to investigate the interaction of soil temperature at the time of application, metam sodium rate, and depth of injection on efficacy of metam sodium in reducing soil populations of *V. dahliae* and the concomitant level of disease management of Verticillium wilt.

Materials and Methods

Trial Location and Field Layout

Trials were conducted under large-scale conditions in irrigated commercial potato production fields located in west central Minnesota in 2010–2011 (hereafter referred to as 2011) and 2011–2012 (2012). The soil in both fields was loamy sand with an organic matter content of 1.3 %. These locations were selected because of a prior history of Verticillium wilt. The fields are on a 3-year potato rotation: 2011 field trial: 2011:potato – 2010:edible beans - 2009:corn – 2008:potato – 2007:edible beans – 2006:corn – 2005:potato and the 2012 field trial: 2012:potato – 2011:edible beans – 2010:corn – 2009:potato – 2008:edible beans – 2007:corn – 2006:potato.

The effects of fumigation injection depth, metam sodium rate and soil temperature at time of injection on the soil populations of *V. dahliae*, subsequent wilt development, yield, tuber quality and stem colonization were assessed using a 2×2×5 factorial arrangement of treatments in a randomized split strip-block design with two replicates. Soil temperature at application was the main blocking factor, metam sodium injection depth was randomized in the split strips, and fumigation rates were randomly nested as subplots within the soil temperature/injection depth strips, in each of two replicates. Parallel strips were treated with metam sodium (sodium methyldithiocarbamate; Vapam HL ™ = metam sodium – 42 % active, Amvac Chemical Corporation) via shank injection at a single depth of 25 cm or split 50:50 in a dual application at depths of 15 cm and 25 cm using commercial fumigation equipment. The injection implement held 20 shanks spaced 30 cm apart with 25 cm sweeps. When metam sodium was applied at two depths, each shank was equipped with a TeeJet 8003 XR nozzle positioned at 15 cm and a TeeJet 8004 XR nozzle at 25 cm. TeeJet 8006 XR nozzles were used for injection at the single depth of 25 cm. All nozzles distributed fumigant in an 80° fan pattern. Fumigant was applied under 8.7 kPa pressure with the injection implement traveling at a rate of 10.5 km/h. Soil moisture at the time of application in the upper 30 cm was determined to be approximately 80–85 % field capacity using a hand method for estimation (Anonymous 1998). The soil was ripped to a depth of 45 cm with a DMI chisel plow prior to fumigation at rates of 0 (non-treated), 373, 467, 561, and 655 l/ha. For the 2011 field trial, fumigation operations were carried out on September 29, 2010 at a soil temperature of 15 °C at the 15 cm depth and on November 5, 2010 at a soil temperature of 4 °C. The four 610 m×6.1 m injection depth×soil temperature strips were divided into 121.9 m×6.1 m sections with each receiving a different rate of metam sodium. In the fall of 2011, in preparation for the 2012 field trial, each of the five fumigation rates were applied in randomized 152.4×12.6 m sections within 762 m×12.6 m strips. Early fumigation was conducted on October 1, 2011 with soil at 13 °C at a 15 cm soil depth and late injection was completed on November 3, 2011 at a soil temperature of 4 °C. In both years, a water "seal" of approximately 1.5 cm was applied to the fields via the pivot irrigator immediately following the early metam sodium applications to limit off-gassing and volatilization of MITC. This procedure could not employed with the late fumigations because the irrigation equipment had been decommissioned for the season by that time to prevent freeze damage.

Soil Sampling and Analysis

Soil sampling sites were selected along linear transects within and parallel to the direction of the fumigant applications. A total of 200 sites (5 sites×2 reps×20 treatments=200 total plants per year) were sampled each year to establish baseline *V. dahliae* populations prior to fumigation and soil from the same sites was sampled again following metam sodium injection. Each site was marked with a flag so it could be revisited for further sampling and data collection. Ten soil samples were collected at arbitrary locations within a 1 m radius around each site-marking flag using a 2 cm diameter soil core probe. Samples were separated into upper (0 to 10 cm) and lower (10 to 20 cm) strata and bulked at each site. Pre-fumigation sampling from the flagged sites was conducted on September 28, 2010 and September 30, 2011. Post-fumigation samples were collected on May 24, 2011 prior to crop emergence and on April 5, 2012 prior to planting. Soil samples were air-dried at 25 °C for 3 to 4 days and stored at 15 °C prior to analysis and *V. dahliae* was quantified as Verticillium propagules per gram of soil (Vppg) (Pest Pros, Inc., Plainfield, WI, USA; Nicot and Rouse 1987a).

Fumigation Efficacy—*Verticillium* Soil Populations and Wilt Assessment

The effect of metam sodium on the *V. dahliae* populations was assessed quantifying the number of Vppg in the upper and lower soil strata at each sampling site following shank injection of the fumigant. Fields were planted to Russet Burbank, a potato cultivar moderately susceptible to *V. dahliae* (Pasche et al. 2013b; Rowe and Powelson 2002), on May 3, 2011 and April 26, 2012. Seed was spaced within rows at 0.36 m with 0.91 m between rows. Agronomic practices commonly used in commercial potato production in the region were employed throughout the season by the cooperating grower. Severity of Verticillium wilt was monitored at approximately 7-day intervals during each growing season commencing on July 28 in 2011 and July 3 in 2012 by estimating the percentage of the canopy within each soil sampling site showing chlorosis, necrosis and/or wilt typical of *V. dahliae* infections. Data were transformed to area under the wilt progress curve (AUWPC) according to the method outlined by Shaner and Finney (1977). AUWPC values were normalized by dividing them by the graph's total area and the resulting relative area under the wilt progress curve (RAUWPC) was used to compare treatments (Fry 1978).

Post-Harvest Evaluations

Prior to vine kill by mechanical flailing on September 12, 2011 and September 18, 2012, one plant was collected from each of the 20 predetermined sites where wilt was evaluated. Dried stem tissues were trimmed to 10 cm lengths, starting 2.5 cm above the soil line, and ground using a Wiley mill equipped with a 40 mesh screen (Pasche et al. 2013a). In 2011, *V. dahliae* was quantified via traditional plating assays by placing a 50 mg sub-sample of processed stem tissue on solid Sorenson's NP-10 medium (Farley 1972) and incubating for 4 to 5 weeks in the dark. Running tap water was used to wash dried stem tissue from the agar surface and plates were allowed to dry overnight prior to examination using a stereomicroscope under 60× magnification. Colonies were identified as *V. dahliae* based on morphological characteristics of microsclerotia and characteristic whorled conidophores on hyaline hyphae and the number of colony forming units (CFU)/g dried stem tissue was calculated. Previous results indicated that QPCR provided results which correlated well with those obtained from much more labor intensive plating techniques, therefore, in 2012 QPCR was performed to quantify the host:pathogen interaction in dried stem tissue (Pasche et al. 2013b). Target DNA in the *V. dahliae* trypsin protease gene was amplified in a 25 ul duplex QPCR reaction utilizing the Vtp1-2 primers and probe (Pasche et al. 2013a). The PotAct primers and probe were used as an internal reference to amplify a target in the potato actin gene. DNA was extracted from 15 mg of a sub-sample of processed stem tissue previously pulverized for 45 s using an MP FastPrep-24 following the manufacturer's instructions for the FastDNA Spin Kit (MP Biomedicals). Quantification was achieved by determining the proportion *V. dahliae* DNA to host DNA based on relative quantification threshold values (Pasche et al. 2013a).

A single 7.6 m row centered on each site marking flag extending through the site specific sampling area was harvested on September 14, 2011 and September 20, 2012 and total yield was determined on a tuber fresh weight basis. French fry processing grade was determined for tubers combined by replication by the USDA Agricultural Marketing Service, Minnesota Department of Agriculture at the USDA Grading Facility - Lamb Weston/RDO Frozen, Park Rapids, MN on September 24, 2011 and October 27, 2012. Tubers were held in a potato storage shed at the facility prior to grading.

Statistical Analysis

Vppg in the upper and lower soil strata, Verticillium wilt severity represented by RAUWPC, total and market yield, tuber quality, and stem colonization from each year were analyzed using Proc Mixed of SAS at $\alpha=0.05$. Analyses of variance (ANOVA) were performed and t tests were used to identify differences in all parameters, soil temperature at the time of fumigation (main strip block), fumigation depth (split-strip) and metal sodium application rate (sub plot). Linear regression analyses ($n=20$) across metam sodium rates were performed to determine the effect of soil *V. dahliae* levels on RAUWPC, both total and marketable yield, and stem colonization Additionally, linear regressions were performed to determine the effect of RAUWPC on total, and marketable yield as well as stem colonization on RAUWPC and total and marketable yield.

Results

Evaluation of Soil *V. dahliae* Levels

Results from the analysis of the 2011 field trial revealed a significant interaction between injection depth × metam sodium rate in soil samples obtained at 0 to 10 cm ($P=0.0351$), however, this interaction was determined to be a result of initial *V. dahliae* infestation levels. The interaction was no longer significant when the zero metam sodium rate, or non-treated control, was removed from the analysis ($P=0.0964$; data not shown) and no interactions were observed at 10 to 20 cm, therefore, further analyses were performed on the main effects. A significant difference was observed in Vppg at the 0 to 10 cm ($P=0.0008$) and 10 to 20 cm ($P<.0001$) soil sampling depths among metam sodium application rates in 2011. At both soil sampling depths, Vppg in the non-treated control were significantly higher than all metam sodium injection rates, but there were no significant differences among metam sodium rates (Table 1).

In 2012, significant interactions were observed for soil temperature at the time of injection × metam sodium rate ($P=0.0110$) and soil temperature at the time of injection × metam sodium rate × injection depth ($P=0.0002$) for Vppg collected at a depth of 0 to 10 cm. When the non-treated control was removed from the analysis these interactions were no longer significant ($P=0.2876$; $P=0.6433$; data not shown). Similar to what was observed in 2011, across injection depths and temperatures, Vppg at 0 to 10 cm were significantly lower when metam sodium was applied at any rate evaluated ($P<.0001$; Table 2). The highest metam sodium rate of 655 l/ha resulted in a significantly lower level of Vppg compared to the 373 l/ha rate, but rates of 467 and 561 l/ha were not significantly different than the lowest or highest rate. Also in 2012, significant interactions of metam sodium rate × injection depth ($P=0.0299$) and a significant three-way interaction of metam sodium rate × injection depth × soil temperature at the time of injection ($P<0.0001$) for Vppg in the 10 to 20 cm sampling depth remained significant, even after the non-treated control was removed from the analysis ($P=0.0323$; $P=0.0141$). In the two-way interaction, variable differences were observed between injection depth at each rate, however, these differences were not significant at any rate (Table 3). The three-way interaction was attributed to the relatively low Vppg level in the non-treated field plots and subsequent lack of significant reduction in Vppg when metam sodium was injected at two depths at 13 °C and at 373 l/ha and 561 l/ha (data not shown). Across injection depths and soil temperatures, all rates of metam sodium decreased Vppg at 10 to 20 cm when compared to the non-treated control ($P<.0001$), but as was observed in 2011, no differences were observed among metam sodium rates (Table 2).

Across both years the study was performed, metam sodium was more effective at reducing *V. dahliae* infestation levels at lower soil depths. In 2011, injections at all rates of metam sodium evaluated in this study resulted in reductions of Vppg at 0 to 10 cm from 20 to 38 %. At the 10 to 20 cm sampling depth, Vppg reductions ranged from 55 to 72 %, depending on the rate, when compared to the non-treated control. Additionally, prior to fumigation, 70 % of all pathogen propagules detected in the 0 to 20 cm soil profile were located in the top 10 cm. After soils had been fumigated with metam sodium, the proportion of *V. dahliae* in the top 10 cm ranged from 80 to 84 %, depending on metam sodium rate. The effect of metam sodium on distribution of the pathogen through the soil profile was similar in 2012. Reductions in Vppg, ranged from 34 to 54 % at 0 to 10 cm and 72 to 84 % at 10 to 20 cm compared to the non-treated control. Prior to soil fumigation, the 0 to 10 cm soil stratum contained 57 % of the *V. dahliae* propagules but after metam sodium fumigation, the upper soil stratum contained between 71 and 79 % of the total. The main effects of soil temperature at the time of injection and injection depth, had very little effect on the efficacy of metam sodium (Fig. 1). Soil temperature at the time of injection was significant only in 2012 in the 0 to 10 cm soil stratum where *V. dahliae* levels were reduced when metam sodium was applied at 4 °C than at 13 °C ($P=0.0461$; Fig. 1c). Additionally, soil infestation was numerically very similar (within 2 Vppg) and,

Table 1 Effect of metam sodium rate on *Verticillium dahliae* propagules per gram of soil (Vppg) at two depths, relative area under the wilt progress curve (RAUWPC), yield, USDA grade and colony forming units per gram of dried stem tissue for the 2011 field trial

Metam sodium rate (l/ha)	Vppg		RAUWPC	Total yield (mt/ha)	Market yield (mt/ha)	Tubers greater than 283 g (%)	Tubers greater than 170 g (%)	Tubers less than 113 g (%)	Unusable tubers (%)	Stem colonization (CFU/g)
	0 to 10 cm	10 to 20 cm								
Nontreated	33.80 a	14.15 a	0.418 a	48.06 b	36.17 b	3.90 a	28.22 b	47.06 a	24.72 a	23870 a
373	27.15 b	6.30 b	0.335 b	51.30 a	41.14 a	6.74 a	34.28 a	45.87 a	19.86 b	15927 c
467	22.70 b	4.90 b	0.296 bc	52.06 a	41.70 a	6.22 a	32.84 ab	47.22 a	19.94 b	18073 bc
561	20.90 b	3.95 b	0.303 bc	51.28 a	41.54 a	8.83 a	36.12 a	46.72 a	19.01 b	20742 ab
655	23.95 b	5.90 b	0.267 c	51.90 a	42.44 a	7.98 a	36.72 a	45.16 a	18.12 b	19907 abc
P value	0.0008	<.0001	<.0001	0.0003	0.0046	0.0968	0.0360	0.6431	0.0016	0.0220

Values in a column followed by the same letter are not statistically different based on Fisher's protected least significant difference (α=0.05). *P* value represents the probability of observing a greater value in the *F* test

[Y] Stem colonization was quantified as *V. dahliae* colony forming units per gram of stem tissue (CFU/g) using traditional plating techniques

therefore, not significantly affected by metam sodium injection depth at either soil sampling depth in 2011 or 2012 (data not shown).

Verticillium Wilt Control with Metam Sodium

In 2011, a significant interaction between metam sodium rate × soil temperature at injection was observed for symptoms of Verticillium wilt, as measured by RAUWPC (*P*=0.0003). Unlike the results for *V. dahliae* soil infestation levels, this interaction could not be explained by the reaction in the non-treated control compared to metam sodium injection treatments (*P*=0.0014). Rather, it was likely due to a significant reduction in wilt severity observed from 15 °C to 4 °C at 467 l/ha, while differences in wilt severity between the two application temperatures at all other rates were not significant (Table 4). No differences were observed in RAUWPC within the main effects of soil temperatures at the time of injection or injection depth (data not shown). However, symptoms of Verticillium wilt were significantly less severe where metam sodium was injected compared to the non-treated control (*P*<.0001; Table 1). The high rate of 655 l/ha controlled Verticillium wilt significantly better than the low rate of 373 l/ha, but not significantly better than 467 and 561 l/ha. Significant interactions in RAUWPC were observed in 2012 for metam sodium rate × injection depth (*P*=0.0114) and metam sodium rate × soil temperature (*P*<.0001). These interactions also could not be explained by the non-treated control (*P*=0.0194; *P*=0.0116). However, cooler soil temperatures (4 °C) at the time of metam sodium application, and a single application depth of 25 cm, resulted in better control of Verticillium wilt severity across all metam sodium rates compared to metam sodium injection at 15/25 cm and warmer (13 °C) soil temperatures, respectively. This is demonstrated by the significant difference observed between the main effects of soil temperature at application where injections at 4 °C resulted in plants with significantly (*P*=0.0315) less wilt (RAUWPC=0.11) than injections conducted at 13 °C (RAUWPC=0.18). The overall severity of Verticillium wilt was substantially lower in 2012, however, the trends in disease control were similar

Table 2 Effect of metam sodium rate on *Verticillium dahliae* propagules per gram of soil (Vppg) at two depths, relative area under the wilt progress curve (RAUWPC), yield, USDA grade and colonization of dried stem tissue for the 2012 field trial

Metam sodium rate (l/ha)	Vppg		RAUWPC	Total yield (mt/ha)	Market yield (mt/ha)	Tubers greater than 283 g (%)	Tubers greater than 170 g (%)	Tubers less than 113 g (%)	Unusable tubers (%)	Stem colonization (mg/g)
	0 to 10 cm	10 to 20 cm								
Nontreated	18.85 a	14.13 a	0.297 a	54.48 b	43.75 b	13.02 b	48.33 b	31.89 a	19.78 a	432712 a
373	12.40 b	3.65 b	0.120 b	63.74 a	56.77 a	21.53 a	60.72 a	28.16 b	11.12 b	260345 b
467	10.80 bc	3.50 b	0.110 b	65.06 a	57.85 a	25.70 a	63.10 a	25.72 b	11.18 b	136132 cd
561	9.75 bc	3.90 b	0.119 b	63.80 a	56.83 a	24.29 a	60.67 a	28.07 b	11.27 b	182220 c
655	8.75 c	2.30 b	0.084 c	63.95 a	57.20 a	24.02 a	61.99 a	27.86 b	10.87 b	104756 d
P value	<.0001	<.0001	<.0001	<.0001	0.0004	0.0003	0.0003	0.0152	<.0001	<.0001

[Y] Stem colonization was quantified as milligrams of *V. dahliae* per gram of stem tissue (mg/g) using the QPCR assay

Values in a column followed by the same letter are not statistically different based on Fisher's protected least significant difference (α=0.05). *P* value represents the probability of observing a greater value in the *F* test

Table 3 Colony forming units per gram of host stem tissue (CFU/g) in 2011 and *Verticillium dahliae* propagules per gram of soil (Vppg) at 10 to 20 cm, relative area under the wilt progress curve (RAUWPC) and total yield evaluated in the 2012 field trial on non-treated and four rates of metam sodium applied in at two injection depths

Metam sodium injection depth (cm)	Metam sodium rate (l/ha)	2011	2012		
		CFU/g	Vppg (10–20 cm)	RAUWPC	Total yield (mt/ha)
25	Non-treated	25701 a	15.6 a	0.301 a	490.2 c
15+25	Non-treated	22040 a	12.7 a	0.293 a	480.2 c
25	373	14658 b	3.0 bc	0.100 cd	558.2 b
15+25	373	17196 b	4.3 bc	0.139 bc	577.3 ab
25	467	16663 b	3.9 bc	0.104 cd	562.0 ab
15+25	467	19483 ab	3.1 bc	0.117 cd	597.0 a
25	561	26519 a	2.2 bc	0.080 d	576.0 ab
15+25	561	14965 b	5.6 b	0.157 b	560.5 b
25	655	20819 a	2.6 bc	0.073 d	577.8 ab
15+25	655	18994 b	2.0 c	0.096 d	561.4 b
P value		0.0204	0.0299	0.0114	0.0472

Means separated by the same letter are not significantly different according to Fisher's protected least significant difference (α=0.05). *P* value represents the probability of observing a greater value in the F test

between the 2 years. In 2012, all rates of metam sodium controlled wilt more effectively than the non-treated control (P<.0001), and 655 l/ha provided significantly better control compared to lower metam sodium rates (Table 2).

Impact of Metam Sodium Applications on Potato Yield

In 2011, no interactions between main effects were observed for total yield, market yield or any tuber quality parameters. Fumigation with metam sodium significantly increased total (P=0.0003) and market yield (P=0.0046) compared to the non-treated control, however, as has been observed with other parameters, increasing metam sodium rates did not significantly affect either total or market yield (Table 1). Among the parameters measured by USDA grade, the injection of metam sodium significantly decreased the percentage of unusable tubers at all rates (P=0.0016) and significantly increased the percentage of tubers greater than 170 g at all rates except 467 l/ha compared to the non-treated control (P=0.0360). While the application of metam sodium also numerically

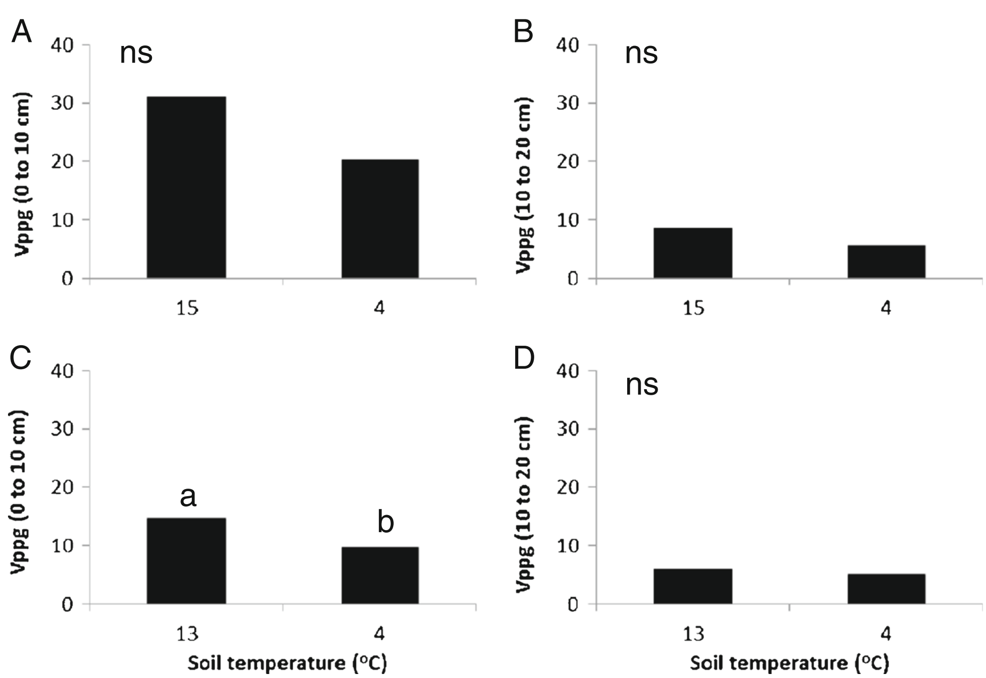

Fig. 1 (5). *Verticillium dahliae* propagules per gram of soil (Vppg) at 0 to 10 (**a**, **c**) and 10 to 20 cm (**b**, **d**) below the soil surface in 2011 (**a**, **b**) and 2012 (**c**, **d**) following metam sodium application at two soil temperatures

Table 4 Wilt severity as measured by relative area under the wilt progress curve (RAUWPC) and stem colonization by *Verticillium dahliae* evaluated in the 2011 as colony forming units per gram (CFU/g) via traditional plating and 2012 as mg *V. dahliae*/g stem tissue via real-time QPCR assays field trials on non-treated (NTC) and four rates of metam sodium applied at two soil temperatures

Soil temperature (°C)[a]	Metam sodium rate (l/ha)	RAUWPC		*V. dahliae* CFU/g	*V. dahliae* (mg/g)
		2011	2012	2011	2012
15/13	Non-treated	0.398 ab	0.300 a	28328 a	405830 a
4	Non-treated	0.438 a	0.294 a	19412 bc	459594 a
15/13	373	0.347 cd	0.185 b	16736 cd	431136 a
4	373	0.324 cde	0.054 ef	15118 cd	89554 cde
15/13	467	0.370 bc	0.140 cd	25822 ab	169487 c
4	467	0.223 fg	0.080 ef	10324 d	102776 cde
15/13	561	0.323 cde	0.164 bc	21192 bc	277248 b
4	561	0.283 def	0.073 ef	20292 bc	87192 de
15/13	655	0.267 efg	0.127 d	19882 bc	167110 cd
4	655	0.268 defg	0.042 f	19931 bc	42402 e
P value		0.0003	<.0001	0.0052	<.0001

Means separated by the same letter are not significantly different according to Fisher's protected least significant difference (α=0.05). *P* value represents the probability of observing a greater value in the F test

[a] Soil temperature at the time of first metam sodium injection was 15oC for 2011 field trials and 13 °C for 2012 trials

increased the percentage tubers greater than 283 g and less than 113 g, these differences were not significant (P=0.0968; P=0.6431).

The interaction of metam sodium rate × injection depth (P=0.0472) and a three way interaction of metam sodium rate × soil temperature × injection depth (P=0.0349) were significant for total yield in 2012. When the non-treated control was removed from the analysis, the three-way interaction was no longer significant (P=0. 1851; data not shown), but this did not effectively explain the interaction of metam sodium rate × injection depth (P=0. 0436). At the lower metam sodium rates, total yield was numerically higher with injection at 15/25 cm, compared to a single injection at 25 cm, however, at higher application rates, metam sodium applied at a single injection depth of 25 cm resulted in numerically higher yields (Table 3). A significant three-way interaction of soil temperature at application × injection depth × metam sodium rate (P=0.0323) also was observed in the percentage of unusable tubers, but this interaction was not significant when the non-treated control was removed from the analysis (P=0.1149; data not shown). As was observed in 2011, fumigation with metam sodium significantly increased total (P<.0001) and market (P=0.0004) yield compared to the non-treated control in 2012, however, as has been observed with other parameters, increasing metam sodium rates did not significantly affect either total or market yield (Table 2). Additionally, the percentage tubers greater than 283 g (P=0.0003) and 170 g (P=0.0003), percentage tubers less than 113 g (P=0.0152) and percentage of unusable tubers (P<.0001) were significantly different in the non-treated control plots than in plots with any rate of metam sodium applied (Table 2). No significant differences were observed among metam sodium rates for any yield or grade parameter evaluated. Injection depth of metam sodium and the soil temperature at the time of injection did not significantly affect any yield or grade parameters measured in either year of this study (data not shown).

Colonization of Potato Stems by *V. dahliae*

In 2011, where stem colonization was evaluated using traditional plating of dried stem material, significant interactions were observed between the main effects of injection depth × metam sodium rate (P=0.0204) and rate × temperature at injection (P=0.0052). These interactions remained significant in the absence of, and could not be attributed to, the non-treated control (P=0.0099; P=0.0036). The rate × injection depth interaction was attributed to the lack of significant difference between a single and two injection depths at lower temperatures, while injecting at two depths provided significantly better control of host colonization at higher metam sodium rates (Table 3). The rate × soil temperature interaction was attributed to significant differences between soil temperatures at 467 l/ha, but not at other rates (Table 4). Across injection depths and soil temperatures, significant differences in stem colonization were observed across metam sodium rates (P=0.0220), however, not all rates significantly reduced colonization, as was observed with all other parameters evaluated in these trials (Table 1). Stem colonization in the lower rates of 373 and 467 l/ha were significantly lower than the non-treated control, but levels in the 561 and 655 l/ha rates were not. No significant difference was observed for the main effects of temperature at injection (P=0.1255) and injection depth (P=0.4675).

In 2012, when host colonization was measured using QPCR, an interaction between metam sodium rate × injection timing was significant for stem colonization (P<.0001). This interaction could not be explained by the non-treated control (P<.0001), however, while lower (4 °C) temperatures at the time of metam sodium injection resulted in lower levels of stem colonization by *V. dahliae* the difference was not significant at 467 l/ha (Table 4). Across both injection depths and soil temperatures, all rates of metam sodium significantly reduced stem colonization when compared to the nontreated control (P<.0001). Application at the lowest rate of 373 l/ha resulted in significantly more host colonization than higher rates, and application at the highest rate of 655 l/ha resulted in significantly lower host colonization than all rates except 467 l/ha (Table 2). Additionally, host colonization was significantly lower (P=0.0192) when metam sodium was injected at 4 °C (156,304 mg *V. dahliae*/g stem tissue) compared to 13 °C (290,162 mg *V. dahliae*/g stem tissue).

Effect of Metam Sodium Rate on the Management of Verticillium Wilt

In 2011, a significant proportion of the total variation in RAUWPC and potato yield was predicted by *V. dahliae* soil infestation levels, based on linear regression analyses (Table 5). While the level of Vppg at 0 to 10 cm had a significant effect on RAUWPC, it was not a good predictor, explaining only 26 % of the variation observed in RAUWPC (R^2=0.2632; P=0.0207). However, the level of Vppg was more predictive of RAUWPC at the lower soil depth of 10 to 20 cm (R^2=0.4477; P=0.0013). Vppg at 0 to 10 cm did not significantly predict total yield (R^2=0.1042; P=0.1650) however, the level of Vppg at 10 to 20 cm significantly predicted 55 % of the variation in total yield (R^2=0.5491; P=0.0002). A similar situation existed with market yield where the variation was not predicted by Vppg at 0 to 10 cm (R^2=0.1561; P=0.0847) but 68 % of the variation was significantly explained by Vppg at 10 to 20 cm (R^2=0.6804; P<.0001). Variation in total yield (R^2=0.2030; P=0.0462) and market yield (R^2=0.3753; P=0.0041) also were significantly predicted by RAUWPC, but only 20 % and 38 % of the variation was explained, respectively (data not shown). Host colonization was not significantly predicted by *V. dahliae* populations in either the upper (R^2=0.0345; P=0.4328) or lower (R^2=0.0723; P=0.2517) soil depths. Additionally, stem colonization did not predict wilt severity (R^2=0.1649; P=0.0757), total (R^2=0.0001; P=0.9857) or marketable yield (R^2=0.0311; P=0.4570).

In 2012, *V. dahliae* soil infestation levels more effectively explained variation in RAUWPC and yield and these predictions were more commonly significant. Vppg at 0 to 10 cm (R^2=0.6441; P<.0001) and 10 to 20 cm (R^2=0.7427; P<.0001) significantly predicted 64 % and 74 % of the variation in RAUWPC, respectively. Total yield also was significantly predicted by Vppg at 0 to 10 cm (R^2=0.7007; P<.0001) and 10 to 20 cm (R^2=0.6335; P<.0001). Market yield was predicted to a greater degree than total yield by Vppg at 0 to 10 cm (R^2=0.7428; P<.0001) and 10 to 20 cm (R^2=0.7092; P<.0001). Also in 2012, RAUWPC predicted total (R^2=0.7232; P<.0001) and market (R^2=0.7608; P<.0001) yield to a much greater extent than observed in 2011 (data not shown). Sixty-eight and 63 % of stem colonization as measured by QPCR was significantly predicted by Vppg in the upper

Table 5 Regression analyses among relative area under the wilt progress curve (RAUWPC), total and market yield, *Verticillium dahliae* propagules per gram of soil (Vppg) and stem colonization evaluated in 2011 and 2012

		Vppg					
		2011			2012		
		0 to 10 cm	10 to 20 cm	0 to 20 cm	0 to 10 cm	10 to 20 cm	0 to 20 cm
RAUWPC	R^2	0.2632	0.4477	0.3676	0.6441	0.7427	0.7749
	P	0.0207	0.0013	0.0046	<.0001	<.0001	<.0001
Total yield	R^2	0.1042	0.5491	0.2523	0.6841	0.6132	0.7412
	P	0.1650	0.0002	0.0240	<.0001	<.0001	<.0001
Market yield	R^2	0.1561	0.6804	0.3408	0.7285	0.6931	0.8097
	P	0.0847	<0.0001	0.0069	<.0001	<.0001	<.0001
Host colonization[a]	R^2	0.0345	0.0723	0.0527	0.6753	0.6283	0.7368
	P	0.4328	0.2517	0.3303	<.0001	<.0001	<.0001

[a] 2011 host colonization was determined using traditional agar plating techniques. Real-time QPCR assay was used for quantification of host colonizations by *V. dahliae* in 2012

(R^2=0.6753; P<.0001) and lower (R^2=0.6283; P<.0001) soil layers, respectively. Contrary to results from 2011, nearly 88 % of wilt severity was predicted by host colonization (R^2=0.8781; P<.0001) and 61 % and 67 % of total (R^2=0.6123; P<.0001) and marketable (R^2=0.6747; P<.0001) yield also were predicted. These relationships proved to be a much stronger than observed for colonization as measured using traditional plating techniques in 2011.

Discussion

Verticillium wilt, as the principal component of the early dying complex in the U.S., is arguably the most economically important disease of the potato crop on an annual basis when direct losses due to the disease and the cost of control are considered (Rowe and Powelson 2002). The U.S. potato industry continues to be challenged by an ever-changing set of production parameters that increasingly demand improved tuber quality despite lower profit margins. Potato production practices also continue to be placed under increased scrutiny by food processors and the consumer which demand safer food supplies from an agricultural industry, while using cultural practices that are more environmentally conscious. The re-registration of metam sodium increased the restrictions on the application of the fumigant which are directed primarily at reducing volatile emissions and non-target exposure. This is only one example of the external pressures and challenges that directly impact the U.S. potato industry since metam sodium remains one of the principal means by which Verticillium wilt is managed (Rowe and Powelson 2002; Taylor et al. 2005). Metam sodium is the most widely used soil fumigant in the U.S. and the potato industry uses approximately one-half of the fumigant manufactured, with 90 % of that used in Idaho, Oregon, Washington and California (Cox 2006). The data reported here provide valuable information that should improve efficacy of metam sodium for the control of Verticillium wilt, potentially improve sustainability of potato production, while also significantly reducing the risk of volatile emissions and non-target exposure of this toxic biocide.

In an earlier study, this research group determined that approximately three-fourths of *V. dahliae* soil propagules resided in the upper 0–10 cm soil stratum but were only reduced by about 20 % after fumigation with metam sodium (Taylor et al. 2005), presumably due to the rapid loss of MITC in the upper surfaces that reduced efficacy. Reductions of *V. dahliae* in the soil were always significantly higher in the lower soil stratum of 10 to 20 cm regardless of method of metam sodium application. This initial study also demonstrated that moldboard plowing, as opposed to conventional shank tillage methods, could significantly redistribute *V. dahliae* from the upper 0 to 10 cm stratum to the 10 to 20 cm stratum and improve metam sodium efficacy (Taylor et al. 2005). In the current study, *V. dahliae* microsclerotia were similarly distributed with approximately 60 to 70 % found in the upper 0 to 10 cm stratum. It is interesting to note that in 2011 levels of Vppg in the lower 10 to 20 cm soil stratum more highly influenced Verticillium wilt development, total yield and marketable yield compared to the levels of Vppg in 0 to 10 cm stratum. Although the comparisons of Vppg in the upper and lower stratum with wilt development and yield in 2012 did not clearly indicate that *V. dahliae* in the 10 to 20 cm stratum to be of greater importance than the upper 0 to 10 cm stratum, it did cause us to review the data published from an earlier study (Taylor et al. 2005). When these earlier data from a field with the same soil type as the fields used in the current study were reanalyzed (n=8) we found that the population of *V. dahliae* in the lower soil stratum (10 to 20 cm) of potatoes grown under a 2 year rotation significantly predicted the level of wilt severity (R^2=0.6977, P=0.0098) and total yield (R^2=0.6031, P=0.0234) when compared to the population of the fungus in the upper soil stratum (0 to 10 cm), where wilt (R^2=0. 3030, P=0.1574) and yield (R^2=0. 4496, P=0.0688) were not significantly predicted. However, under a 3 year potato rotation, *V. dahliae* levels did not predict wilt severity (R^2=0.2045, P=0.2606; R^2=0.3923, P=0.0966) or total yield (R^2=0.0246, P=0.7109; R^2=0.1012, P=0.4426) in the lower or upper soil stratum, respectively. These data suggest that although soil inoculum potential of *V. dahliae* may be lower in the 10 to 20 cm soil stratum compared to the 0 to 10 cm stratum, the inoculum in the lower soil levels may be more efficient and, therefore, may be more important from a disease management perspective. This may be due to such factors as more uniform moisture that likely increases microsclerotial germination or higher potato root density in the lower soil profile than near the soil surface. Previous studies have shown that higher soil moisture increases *V. dahliae* colonization and wilt development (Cappeart, et al. 1992). Additionally, a higher density of roots emerges from the first node above the seed piece and nodes above this produce predominately adventitious roots (Loria 2001) which function more for support than for water and mineral uptake (DeRoo and Waggoner 1961). Potato root density in the 19 to 40 cm profile of a sandy loam soil, as determined by root weight, has been demonstrated to be 2× to 14× that of the 0 to 19 cm profile but can also be influenced by such factors as soil compaction and method of tillage (DeRoo and Waggoner 1961). In other words, although *V. dahliae* inoculum potential may be lower in the 10 to 20 cm soil profile, as evidenced by the data presented here and elsewhere (Taylor et al. 2005), more consistent soil moisture and higher potato root density in this soil stratum may result in higher inoculum efficiency of *V. dahliae* compared to the 0 to 10 cm stratum. Thus, it is evident that we were incorrect in our earlier assumptions that more effective Verticillium wilt control can be obtained by focusing on methods that improve the efficacy of metam sodium in *V. dahliae* reduction in the upper soil profile. It is apparent that methods of metam sodium application that improve overall efficacy must be evaluated, such as the shank injection depth and the temperature of the soil at the time of application.

There are several important pieces of information regarding factors affecting metam sodium efficacy that potentially can improve Verticillium wilt control derived from the current studies. Perhaps one of the more interesting developments from this research was the inability to detect a consistent rate response in the reduction of *V. dahliae* populations and concomitant reduction in Verticillium wilt severity. Over two growing seasons, soil populations of *V. dahliae* generally varied from approximately 8 to 40 Vppg in the upper 10 cm of soil and from approximately 2 to 15 Vppg in the 10 to 20 cm stratum. Reductions in the soil population were greater in the lower stratum, presumably due to less MITC volatilization compared to the upper soil layers. It has been documented that injection depth has a profound impact on the rate of MITC emission, the deeper the injection the lower the loss due to volatile MITC emission (Saeed et al. 2000; Zhang and Wang 2007; Zheng et al. 2006). Reductions in *V. dahliae* resulting from metam sodium applications in the upper soil stratum varied from 20 to 54 % while in the lower 10 to 20 cm stratum Vppg reductions varied from approximately 55 to 84 %. These results are consistent with previous research performed by our research group in which overall reductions of the fungus were in the range of 60 to 80 % but were only 0 to 25 % in the upper soil profile (Taylor et al. 2005). However, in the earlier study, only a single 373 l/ha metam sodium rate was evaluated. In the current study, no differences in the reduction of soil populations of *V. dahliae* were detected in 2011 among metam sodium rates ranging from 373 to 655 l/ha, although this finding is consistent with previously published data (Hamm et al. 2003), it is nonetheless surprising since *V. dahliae* has been documented to be one of the more difficult soil borne pathogens to kill with this soil fumigant (Klose et al. 2008). It should be noted, however, that in 2012 the high rate of metam sodium used (655 l/ha) did significantly reduce the *V. dahliae* population in the upper soil layer and wilt severity compared to the lowest rate of 373 l/ha, but these reductions did not result in higher potato yields. This overall lack of a metam sodium rate response in reduction of Vppg and Verticillium wilt severity and lack of yield increases is important not only for economic reasons, but should also contribute significantly to reducing MITC emissions and non-target exposure since the rate of metam sodium is a significant factor in conversion efficiency to MITC and in the loss due to volatile emissions (Sullivan et al. 2004; Yates et al. 2002; Zhang and Wang 2007; Zheng et al. 2006). Previously published data have demonstrated a lack of positive yield response through the use of higher rates of metam sodium (Hamm et al. 2003), however, it should be noted also that low rates of 300 l/ha did not significantly increase potato yield in Israel (Tsror et al. 2005).

The data on shank injection depth are also similarly positive. In previous studies we determined that dual shank injection depths of 15/30 cm depths were the least effective method of metam sodium application and inferior to that of 10/25 injection depths and chemigation when conventional "deep rip" pre-fumigation tillage was used (Taylor et al. 2005). Although there were no data to support a modification of shank injecting metam sodium at 15/25 cm depths, this injection method is the most commonly used shank injection method in the Midwest (Gudmestad, unpublished), which is why it was evaluated in this study. It was surprising to find that a single injection of metam sodium at a depth of 25 cm was as efficacious as a 15/25 cm dual injection, particularly in the levels of Vppg in the upper 10 cm. There are numerous advantages to injecting metam sodium at a single depth including less power needed to pull a single sweep shank through the soil rather than dual sweeps at two levels, less fuel required, and fewer problems with plant residue clogging the shank injection implement. More importantly, a single injection depth of 25 cm, thus eliminating the 15 cm injection depth, will presumably reduce MITC emissions as has been previously demonstrated (Saeed et al. 2000; Zhang and Wang 2007; Zheng et al. 2006).

Perhaps the most intriguing result from the present study was that the efficacy of metam sodium shank injected at soil temperatures of 4 °C was equal to or higher than that observed when the soil fumigant was injected at 13 and 15 °C. Although the EPA label for metam sodium mandates soil temperatures at initiation of application in the range of 1.7 to 32 °C, soil fumigation with this chemical in the Midwest generally range from 7 to 24 °C, with most occurring in the range of 10 to 18 °C (Gudmestad, unpublished). Previous studies suggest that applications of metam sodium at temperatures ranging from 2 to 5 °C can be very efficacious as long as the soil is 'wet' (Leistra and Smelt 1974; Saeed et al. 1997) and that applications of metam sodium at 15 °C were three to six times more effective in killing *V. dahliae* microsclerotia than applications at much warmer temperatures of 30 and 35 °C, respectively (Ben-Yephet and Frank 1985). Additionally, metam sodium persisted in the soil at toxic levels for up to 6 weeks at 10 °C whereas the duration was much shorter (2 weeks) at 25 °C (Vanachter and Van Assche 1970). However, the level of efficacy achieved with shank injections of metam sodium at 4 °C in this study was surprising, largely because we were unable to apply a water seal since it was so late in the season and the water had to be turned off. Applications of metam sodium at colder temperatures should also potentially lower MITC emissions and reduce exposure of this chemical to non-targets. Vapor pressure of metam sodium and conversion to

MITC is known to be significantly reduced with temperatures as low as 3 °C (Smelt and Leistra 1974). These temperature-mediated parameters will likely contribute directly to extending the persistence of metam sodium in the soil (Vanachter and Van Assche 1970), and enhancing fumigant efficacy since the rate of decomposition of MITC is also affected by soil temperature (Leistra and Smelt 1974; Smelt and Leistra 1974; Zheng et al. 2006). High soil temperatures tend to cause more rapid decomposition of MITC which presumably reduces efficacy and may explain why efficacy of metam sodium at 4 °C in the studies reported here were generally higher than when the soil fumigant was applied at either 13 or 15 °C.

In summary, the results of studies reported here have the potential to improve the control of Verticillium wilt in potato by providing a template for more effective use of metam sodium that may also reduce MITC emissions. The loss of MITC through volatile emissions is regarded to be due to a number of factors including application method, initial content of metam sodium in the soil, climatic conditions, soil temperature, soil moisture and soil texture characteristics (Saeed et al. 2000; Sullivan et al. 2004; Yates et al. 2002; Zhang and Wang 2007; Zheng et al. 2006). A number of recommendations have been made from those studies to reduce MITC emissions including the use of sub-surface, or shank injections of metam sodium (Saeed et al. 2000; Zhang and Wang 2007; Zheng et al. 2006). The data are very clear, the deeper the injection, the lower the MITC emission. Shank injections have significantly lower MITC emissions than application of metam sodium applied at the surface (Saeed et al. 2000; Sullivan et al. 2004; Zheng et al. 2006). In this study, shank injection of metam sodium at 25 cm was as efficacious as dual injections of 15 and 25 cm. MITC emissions at 25 cm have been shown to be significantly lower than at 15 cm at the same soil temperature (Zhang and Wang 2007). Since a single injection depth of 25 cm was as efficacious as dual injections with one of those being much more shallow (15 cm), it is apparent that the potato industry can abandon injections of the soil fumigant at the upper levels to reduce MITC emissions, while maintaining efficacy. Additionally, since we found little evidence of a rate response for metam sodium in reducing soil populations of *V. dahliae* and in reducing Verticillium wilt severity without a concomitant increase in potato yield, a lower rate of the soil fumigant at 373 l/ha will provide adequate efficacy while also potentially reducing MITC emissions (Zheng et al. 2006). Finally, shank injections of metam sodium at a temperature as low as 4 °C were highly efficacious in the studies reported here and also have the added advantage in reducing MITC emissions (Leistra and Smelt 1974; Smelt and Leistra 1974; Zheng et al. 2006). We believe that the potato industry can take advantage of using the combination of a single shank injection depth (25 cm), relatively low metam sodium application rates (373 l/ha), and low soil temperatures at the time of application (4 °C) to improve efficacy of metam sodium for the control of Verticillium while increasing tuber yield and quality and reducing non-target exposure to MITC. The net result of this research is a more sustainable and environmentally friendly approach to managing the early dying complex of potato.

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