Auburn University
Southern Forest Nursery
Management Cooperative

RESEARCH REPORT 13-01

EVALUATION OF METHYL BROMIDE ALTERNATIVES ON LOBLOLLY PINE PRODUCTION AND SEEDLING QUALITY OVER THREE GROWING SEASONS AT THE PINE HILL NURSERY IN CAMDEN, ALABAMA

by
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INTRODUCTION

Fumigation with methyl bromide (MBr) mixtures has been the most commonly used method for producing high quality, pest-free forest-tree seedlings in the southeastern United States (Jang et al. 1993). Forest nurseries in the United States have relied for many years on MBr soil fumigation to control yellow and purple nutsedge, soil insects, nematodes and soilborne pathogenic fungi. Forest-seedling nursery managers have long recognized the importance of MBr to control these soil-borne pathogens in the production of forest-tree seedlings South et al. 1997). Due to the concern over ozone depletion in the stratosphere, the Montreal Protocol under the Clean Air Act began a phase-out program for MBr use in 2005. Since 1991, finding alternatives for MBr has been a priority for the forest nursery industry. Other materials tested by the Southern Forest Nursery Management Cooperative, without much success, include basamid, hot pepper sauce, steam, hot water, and biologicals. Although it may be difficult to find a soil fumigant alternative that is as broad-spectrum as MBr, the nursery industry realizes the importance of testing new compounds, rates and application techniques.

Loblolly pine (Pinus taeda L.) is the primary species produced in southern forest nurseries (Enebak S.A. 2011) and many soilborne pests can affect its production (Cordell et al. 1989, South and Enebak 2005). These studies report a portion of the USDA – ARS Areawide Pest Management Project for Methyl Bromide Alternatives and are part of a long-term continuing effort by the Southern Forest Nursery Management Cooperative to identify and evaluate soil fumigants against common soil-borne fungi, weeds, and nematodes and their effect on seedling quality of forest-tree seedlings in the southern United States using operational application techniques under normal nursery management practices over a 4-year cropping system. Despite the phase-out of MBr, forest nursery managers still need soil treatments that are comparable to MBr for control of soilborne pests. Information from these studies should be used by nursery managers in the southern US to select an MBr alternative that could be useful in the production of forest-tree seedlings in their nurseries.

MATERIALS AND METHODS

Soil Fumigants. Included in this trial were six soil fumigation treatments that were selected from previous Southern Forest Nursery Management Cooperative fumigation studies and two rates of iodomethane, which had never been tested previously in the production of forest-tree seedlings (Table 1). The fumigants were shank-injected on March 23, 2009. Iodomethane treatments were covered with Virtually Impermeable Film (VIF), while all other treatments were covered with a 1 mm High Density Polyethylene (HDPE) Tarp (AEP Industries Inc.) as a broadcast/flat tarp (Table 2). The experimental area occupied approximately 2.5 ha and the trial was laid out in six nursery sections that consisted of nine seedling beds between the irrigation pipelines with each bed being approximately 170 m x 1.2 m. The experiment was a randomized complete block design with each treatment being 85 m long and replicated four times. Each 9-bed nursery section included 3 soil fumigation treatments (3 nursery beds per treatment). Because of the different plastic requirements, the iodomethane treatments could not be placed with the other soil fumigants and thus, were analyzed as a separate experiment. After fumigation and in each of the three growing seasons (2009-2011), a single family of loblolly pine seed was sown to the area. Seedlings in the trial area were maintained using standard nursery cultural practices (fertilization, irrigation, pest management, etc.) until lifting took place each fall. After lifting the seedlings in the winter (Nov-Dec), the treatment area was left fallow and prepared for sowing the following spring (April).

Seedling Quality and Quantity. The effect of the soil fumigants on seedling densities and growth characteristics were assessed in four subplots (1.2 m x 0.3 m) per treatment plot at 7 wk post-sowing, mid-summer (15 wk post-sowing) and just prior to lifting in the fall (26 wk post-sowing) in 2009, 2010 and 2011, respectively. Twenty-five loblolly pine seedlings per subplot were collected in the fall of each cropping season and returned to the laboratory at Auburn University for analysis. Seedling root collar diameter (RCD), shoot height and seedling dry weight (biomass) were measured for each seedling as well as overall root growth. For root morphology, 10 seedlings per subplot were examined for root length, root surface area, average root diameter and the number of root tips using WinRhizo® software by Regents Instruments Inc., Quebec, Canada.

Soilborne Trichoderma and Nematodes. Throughout the three cropping seasons, soil samples were collected from the center seedling bed of each 3-bed plot: at pre-sowing, post-sowing, mid-summer and just prior to seedling lifting in November of each growing season. Half of each soil sample was plated onto Trichoderma-selective media (Elad *et al.* 1981) to determine soilborne fungi levels and the remaining half was sent to the Soils Laboratory at Auburn University for a quantitative assessment of nematodes.

RESULTS

Seedling Quality and Quantity. At the Pine Hill Nursery in Alabama seedling densities at the end of the first growing season in 2009 were similar across all soil fumigants tested ranging from 210-228 seedling/m^2. Similar results were observed for the 2010 and 2011 growing seasons for the soil fumigants used under the HDPE plastics. In contrast, soils treated with Midas had significantly fewer seedlings than the MBr treatments in 2009. However, by the end of the second and third growing seasons in 2010 and 2011, there were no significant differences in seedling densities for any of the soil fumigants tested, as all treatments gave similar seedling densities as the standard MBr soil fumigation treatment (Table 3).

Differences in seedling root collar diameters (RCD) among the soil fumigants tested at the Pine Hill nursery were observed during the final growing season with MBr treated soils producing significantly larger seedling diameters over all other soil fumigants tested (Table 4). Some of the newer chemistries, Pic+ and DMDS, had seedlings with similar RCD as MBr during the three growing seasons. Seeding RCDs declined for all treatments over the three-year rotation due to the buildup of soilborne pests and weeds over time.

Overall, seedling root architecture and root morphology as measured by root length, surface area, root diameter and root tips were similar for all soil fumigants over the three cropping seasons (Table 5). Generally, first year soil fumigation results in larger seedlings. However, as far as an MBr alternative, all soil fumigant alternatives performed as well as MBr across all the root morphology measurements at this nursery. Like that of RCD and time since soil fumigation, root characteristics tended to decrease over the three growing seasons.

The root weight ratio (RWR) of seedlings grown in the different soil treatments resulted in differences only during the first growing season (Table 6). The RWR is defined as the weight of the roots divided by the total seedling weight with an optimum seedling having a root weight ratio of >27%. Seedlings with a higher RWR have better survival and growth after outplanting. Of all the soil fumigants tested, Chlor 60 had the lowest RWR of all treatments in 2009. The treatments had similar RWR in 2010 and 2011 (Table 6). While none of the treatments resulted in the optimum RWR, a number of factors affect RWR, including the time of lifting, growing density, the time of root pruning, irrigation and fertilization. For these trials, none of the MBr alternatives were detrimental to root growth that could affect seedling survival after outplanting.

Soilborne Trichoderma and Nematodes.

At the end of the each growing season, nursery soils at Pine Hill, Alabama had similar levels of Trichoderma across all soil fumigants tested (Table 7). In other trials, soil fumigants including MBC 70/30, chloropicrin, and especially Midas® significantly reduced Trichoderma levels over that of MBr (Carey *et al.* 2005). Trichoderma is a beneficial soilborne fungus that is used to monitor the sensitivity of the soil micro-organisms to soil fumigation and, therefore, suppression of Trichoderma is considered undesirable when screening soil fumigates.

Over the course of the 3-yr study, each soil fumigant plot was examined six times for both the number and species of nematodes within the soil/seedling interface. Nematode populations within the soil are rarely distributed uniformly across the nursery beds and these studies had a wide range (0-67 nematodes /100 cc soil) in numbers and species for all soil fumigants used

(Table 8). Because of the variability, there were no differences between treatments for any of the soil fumigants tested. Overall, all soil fumigants were effective in eliminating nematode populations the first growing season which were maintained during the 3-yr rotation. There was an increase during the second growing season, but populations did not increase into the third season (Table 8). One of the more troublesome species on seedling production is the stunt nematode (*Tylenchorhynchus claytoni*) which appeared only during the second cropping season. Of the soil fumigants tested, Chlor 60 was the only soil fumigant not to have any nematodes recovered in soil samples over the course of the study at the Pine Hill nursery. Chlor 60 is comprised of 40% 1, 3-dichloropropene (Telone®) which is highly effective against nematodes. It is not surprising then that this compound was the most effective.

DISCUSSION

The primary objective of the USDA Areawide MBr Alternative program is to identify possible alternatives to MBr using large-scale, multi-year trials in soils and conditions throughout the southern U.S. One of the unique aspects of MBr as a soil fumigant is its ability to consistently control weeds, insects, nematodes and fungi across many different growing conditions and nursery soils. Studies conducted within the southern U.S. in forest-tree nurseries have yet to find an MBr alternative that fits all of these characteristics (Enebak *et al.* 2011, 2012). The true test of an MBr alternative is its performance during the second and third growing season where treatment differences usually begin to appear as disease, weed, and nematode pressures increase. Based on these trials, when MBr is no longer available to forest-tree nurseries, those soil fumigants with chloropicrin appear to be the most useful in controlling pests and producing quality seedlings as seedling densities and root characteristics from plots treated with chloropicrin-based products were similar to MBr.

One of the primary reasons for determining the effects of these soil fumigants on root architecture is that a more fibrous root system increases the chance of seedling survival in the field (Hatchell and Muse, 1990; Frampton *et al.* 2002; Davis and Jacobs, 2005). One soil fumigant, DMDS + chloropicrin, was comparable to MBr in RCD and root morphology characteristics and soilborne Trichoderma levels, but had a significant odor problem (i.e. garlic or strong propane) that lasted long into the growing season. The lingering odor with this particular soil fumigant may limit its acceptance by growers as an alternative to MBr. By far the best MBr alternatives tested were Pic+ and Chlor 60, with both soil fumigants controlling soilborne nematodes and producing quality seedlings.

The application of Midas® (iodomethane & chloropicrin) in these trials was the first large-scale use of this compound on the production of forest-tree seedlings in the United States. Considered a drop-in replacement by the US Environmental Protection Agency, the US Department of Agriculture and the European Union, the compound was applied under virtually impermeable film (VIF) at less than half the rates of the other soil fumigants. The reduced active ingredient was done for two reasons: the iodomethane was expensive (twice the cost of chloropicrin per ha), and the impermeable film increased the effectiveness of the compound by limiting the off-gassing. While the compound showed promise with respect to seedling quality and soilborne pest control, Arista Life Science removed Midas® from the North American market in 2012 due to increasing costs and environmental pressures to re-examine the compound's registration in the US.

In addition to the weed pressures, some nursery soils have a history of nematodes reaching levels that affect seedling production (Cram and Fraedrich 2005). For these reasons, 1-3, dichloropropene (Telone®) may need to be used in nurseries with reoccurring nematode pressure. The soil type at the Pine Hill nursery (Lenior silt loam) is heavier than that of most forest-tree nurseries and generally has not resulted in production issues due to nematode populations. Chlor 60 (containing 40% Telone®) had the lowest nematode levels and may be an option for nurseries that have nematode issues in the second growing season. While the seedling densities and root characteristics with chloropicrin were encouraging, one of the potential pitfalls with using 100% chloropicrin at 300 lb/a is the buffer zone restrictions under current soil fumigation practices in the US. If these restrictions limit the use of 100% chloropicrin, then Pic+ with 85% chloropicrin would be the best alternative to MBr. The final decision when selecting an MBr alternative needs to take into consideration the ability of the soil fumigant to work under individual nursery soil conditions and the impact of new soil fumigation rules that come into place in 2012 have on each individual nursery.

Soil fumigation is an effective way to reduce pathogenic soilborne fungi in the nursery that affect seedling production and survival after outplanting. In other trials, the soil fumigants tested were found to be effective in controlling Pythium, Rhizoctonia, and Fusarium when inoculated onto oatmeal (Enebak *et al.* 2012). The wide-spread use of MBr has minimized extensive seedling losses due to soilborne pathogenic fungi. Pythium still can cause damping-off problems in the early spring and is often limited to areas of poor drainage and standing water. The soil-type at the Pine Hill nursery is conducive to damping-off caused by Pythium early in the growing season. Rhizoctonia can appear in nurseries both as root decay and as foliage blight, especially in the second-year crops post fumigation as the fungus increases over the first growing season (Carey and McQuage 2004). What will happen to nurseries 8 years post-MBr ban is unknown, but at least for this 3-yr trial, soilborne pathogens did not appear to affect seedling production.

One of the unique aspects of soil fumigants currently being tested in southern forest nurseries is that they do not completely eliminate beneficial fungi which are needed for seedling growth. In this nursery, the soil fumigants tested did not completely eliminate all soilborne fungi. This is important since previous research has shown that Trichoderma is an important soil borne fungus necessary for proper pine seedling growth (Bailey and Lumsden 1998; Mousseaux *et al.* 1998). In these trials, the population levels of non-target soilborne fungi rebounded quickly with all soil fumigants used as previous Nursery Cooperative research has shown that Trichoderma is not as sensitive to MBr as other soil fumigants (Carey *et al.* 2005; Starkey and Enebak, 2008). In contrast, dazomet, a soil fumigant tested by the Southern Forest Nursery Management Cooperative for several years, significantly reduced the levels of beneficial fungi which remained after two growing seasons (Starkey *et al.* 2006).

While many nursery managers would prefer to use MBr in perpetuity to grow forest-tree seedlings, MBr will eventually be unavailable and each nursery manager will need to identify the best alternative for their nursery conditions. These trials at the Pine Hill Nursery and others indicate that, while not the perfect replacement, seedling production is still possible without MBr if compounds such as chloropicrin are used and managers pay close attention to weed and nematode pests that are less susceptible to chloropicrin than MBr.

MANAGEMENT IMPLICATIONS

Forest-tree nurseries in the United States have relied on methyl bromide (MBr) soil fumigation to control weeds, fungi, and nematodes. Due to the world-wide phase-out of MBr use, finding a soil fumigant alternative for MBr has been a priority for the forest nursery industry. A large-scale study comparing seven fumigants using operational application techniques and normal nursery management practices over three growing seasons was installed to determine the effects of MBr alternatives on seedling quality and quantity. Seedling densities at the end of the first growing season were similar across all soil fumigants tested. The newer chemistries, Pic+ and DMDS had seedlings with similar RCD as MBr during the first and second growing season. Seeding RCDs declined for all treatments over the three-year rotation due to the buildup of soilborne pests and weeds. Seedling root architecture and root morphology were similar for all soil fumigants. At the end of the each growing season nursery soils had similar levels of Trichoderma and nematode populations across all soil fumigants tested. These trials indicate that, while not the perfect replacement in all nursery soils, seedling production is still possible without MBr if compounds such as chloropicrin are used and managers pay close attention to weed and nematode pests that are less susceptible to chloropicrin than MBr.

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Table 1. MBr alternative soil fumigants and rates used in the 2009-2011 area-wide demonstration trial at Camden, AL.

Treatment	Rate (lb/acre)	Plastic[y]	Fumigant Components
Pic+	300	HDPE	85% chloropicrin & 15% solvent
Chlor 100	300	HDPE	100% chloropicrin
MBr	350	HDPE	67% MBr & 33% chloropicrin
MBC 70/30	400	HDPE	70% MBr (98/2) & 30% solvent
Chlor 60	400	HDPE	60% chloropicrin & 40% 1,3-dichloropropene (Telone®)
DMDS + Chlor	70 (gal/acre)	HDPE	79% dimethyl disulfide & 21% chloropicrin
[z]Midas® 98/2	100	VIF	98% iodomethane & 2% chloropicrin
Midas® 50/50	160	VIF	50% iodomethane & 50% chloropicrin

[y] HDPE = High Density Polyethylene; VIF = Virtually Impermeable Film

[z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 2. Site information for the 2009-2011 Area-wide demonstration trial at Camden, AL.

Trial Parameter	Camden, AL
Fumigation date	March 23, 2009
Fumigation type	Shank injected; broadcast/flat tarp
Experimental area	5 acres
Air temperature	61-77 °F
Wind speed	5-10 mph
Soil moisture	7.6%
Soil series	Lenoir silt loam
Plastic in place	14 days

Table 3. Loblolly pine seedling density at lifting over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Seedling Density (m^2)		
Fumigant	**2009**	**2010**	**2011**
MBr	20.8 a[x]	25.0 a	21.0 a
Chlor 100	21.0 a	23.5 a	20.6 a
MBC 70/30	21.1 a	25.7 a	26.0 a
Chlor 60	20.5 a	21.7 a	23.4 a
Pic+	21.2 a	23.2 a	21.9 a
DMDS + Chlor	19.5 a	23.4 a	22.0 a
[y]LSD $_{(0.05)}$	*(2.9)*	*(7.3)*	*(6.0)*
[z]Midas® 50/50	18.9 a	23.5 a	19.4 a
Midas® 98/2	18.2 b	23.5 a	18.6 a
LSD $_{(0.05)}$	*(0.5)*	*(7.0)*	*(3.1)*

[x] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).
[y] Least significant differences are italicized.
[z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 4. Loblolly pine seedling root collar diameter at lifting over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Root Collar Diameter (mm)		
Fumigant	**2009**	**2010**	**2011**
MBr	4.21 a[x]	4.01 a	3.89 a
Chlor 100	4.33 a	4.12 a	3.46 b
MBC 70/30	3.97 a	3.73 a	3.14 b
Chlor 60	4.46 a	4.13 a	3.34 b
Pic+	4.04 a	4.02 a	3.43 b
DMDS + Chlor	4.26 a	4.18 a	3.45 b
[y]LSD $_{(0.05)}$	*(0.60)*	*(0.54)*	*(0.42)*
[z]Midas® 50/50	4.83 a	3.92 a	3.83 a
Midas® 98/2	4.37 a	3.60 a	3.77 a
LSD $_{(0.05)}$	*(1.87)*	*(1.92)*	*(0.93)*

[x] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).
[y] Least significant differences are italicized.
[Z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 5. Loblolly pine seedling root morphology at lifting over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Root Morphology											
	Root Length (cm)			Root Surface Area (cm^2)			Root Diameter (mm)			Root Tips (#)		
Fumigant	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**
MBr	183 a[x]	167 a	138 a	51 a	53 a	44 a	0.89 a	1.02 a	1.02 a	398 a	457 a	299 a
Chlor 100	168 a	164 a	133 a	49 a	55 a	43 a	0.95 a	1.09 a	1.05 a	350 a	377 a	274 a
MBC 70/30	158 a	156 a	112 a	45 a	47 a	36 a	0.93 a	0.97 a	1.03 a	374 a	405 a	242 a
Chlor 60	169 a	163 a	116 a	49 a	52 a	37 a	0.93 a	1.03 a	1.04 a	328 a	381 a	244 a
Pic+	154 a	161 a	126 a	44 a	49 a	39 a	0.95 a	0.99 a	1.01 a	319 a	398 a	262 a
DMDS + Chlor	162 a	185 a	112 a	47 a	57 a	36 a	0.94 a	1.00 a	1.03 a	328 a	440 a	235 a
[y]LSD $_{(0.05)}$	*(27)*	*(38)*	*(32)*	*(9)*	*(16)*	*(9)*	*(0.09)*	*(0.11)*	*(0.08)*	*(74)*	*(123)*	*(69)*
[z]Midas® 50/50	217 a	181 a	149 a	62 a	57 a	47 a	0.94 a	1.00 a	1.02 a	463 a	354 a	298 a
Midas® 98/2	202 a	159 a	145 a	57 a	52 a	46 a	0.92 a	1.04 a	1.04 a	435 a	300 a	313 a
LSD $_{(0.05)}$	*(359)*	*(127)*	*(142)*	*(54)*	*(10)*	*(30)*	*(0.78)*	*(0.67)*	*(0.52)*	*(531)*	*(440)*	*(278)*

[x] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).
[y] Least significant differences are italicized.
[Z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 6. Loblolly pine seedling root weight ratios at lifting over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Root Weight Ratio (%)		
Fumigant	**2009**	**2010**	**2011**
MBr	12.6 ab[x]	15.7 a	14.7 a
Chlor 100	12.4 ab	15.4 a	15.9 a
MBC 70/30	12.4 ab	13.6 a	14.7 a
Chlor 60	11.2 b	15.3 a	14.5 a
Pic+	12.0 ab	14.9 a	15.0 a
DMDS + Chlor	14.0 a	15.1 a	15.1 a
[y]LSD $_{(0.05)}$	*(2.3)*	*(1.9)*	*(2.4)*
[z]Midas® 50/50	13.3 a	16.3 a	14.5 a
Midas® 98/2	17.1 a	17.1 a	16.3 a
LSD $_{(0.05)}$	*(41.3)*	*(1.6)*	*(13.0)*

[x] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).
[y] Least significant differences are italicized.
[Z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 7. Number of *Trichoderma* colony forming units (CFUs) from soils collected over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Trichoderma (CFUs/mg soil)					
	2009		2010		2011	
Fumigant	**June**	**Dec**	**June**	**Nov**	**June**	**Nov**
MBr	82.5 a[x]	101.0 ab	99.0 a	59.0 ab	79.0 a	76.0 a
Chlor 100	59.8 a	179.0 a	111.0 a	38.3 b	95.2 a	76.5 a
MBC 70/30	36.3 a	135.0 ab	112.5 a	82.0 a	80.7 a	76.2 a
Chlor 60	26.3 a	111.8 ab	188.8 a	46.5 b	82.7 a	69.0 a
Pic+	22.5 a	53.5 b	124.3 a	32.5 b	55.0 b	76.8 a
DMDS + Chlor	35.5 a	77.3 ab	92.3 a	32.5 b	92.6 a	97.2 a
[y]LSD $_{(0.05)}$	*(67.4)*	*(109.9)*	*(107.3)*	*(32.2)*	*(17.7)*	*(30.9)*
[z]Midas® 50/50	123.0 a	56.3 a	187.0 a	74.7 a	94.7 a	85.4 a
Midas® 98/2	28.3 b	34.8 a	101.7 b	116.7 a	75.4 a	94.0 a
LSD $_{(0.05)}$	*(51.9)*	*(44.4)*	*(36.3)*	*(368.3)*	*(217.2)*	*(16.1)*

[x] Means (within a column) followed by the same letter are not significantly different based on Duncan's Multiple Range Test ($p \leq 0.05$).
[y] Least significant differences are italicized.
[Z] The Midas® treatments were considered a separate trial from the other soil treatments due to the plastic requirement.

Table 8. Average number of nematodes per 100 cubic centimeters (cc) of soil over three growing seasons (2009-2011) for the Area-wide trial in Camden, AL.

	Nematodes (# per 100 cc of soil)					
	2009		2010		2011	
Fumigant	**June**	**Dec**[x]	**June**	**Nov**[y]	**June**[z]	**Nov**
MBr	0	29	0	0	0	0
Chlor 100	0	0	0	47	69	0
MBC 70/30	0	0	0	65	0	0
Chlor 60	0	0	0	0	0	0
Pic+	0	0	0	12	0	0
DMDS + Chlor	0	26	0	0	0	0
Midas® 50/50	0	17	0	0	0	0
Midas® 98/2	0	28	0	43	0	0

[x] Nematodes recovered in Dec 2009 were stunt nematodes.
[y] Nematodes recovered in Nov 2010 were stubby root nematodes.
[z] Nematodes recovered in June 2011 were spiral nematodes.