From Forest Nursery Notes, Summer 2013

117. © Nitrification in pine tree substrate is influenced by storage time and amendments. Taylor, L. L., Niemiera, A. X., Wright, R. D., Evanylo, G. K., and Thomason, W. E. HortScience 48(1):115-122. 2013.

HORTSCIENCE 48(1):115–122. 2013.

Nitrification in Pine Tree Substrate Is Influenced by Storage Time and Amendments

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Additional index words. loblolly pine, *Pinus taeda*, most probable number, marigold, *Tagetes erecta* 'Inca Gold', nitrate, ammonium, ammonium-N to nitrate-N ratio, pH

***Abstract*. Pine tree substrate (PTS), for container plant production, is a relatively new alternative to the commonly used pine bark and peat substrates. Fertility management requires knowledge of nitrogen transformations in this new substrate. The objective of this study was to document the occurrence of nitrification in PTS and to determine if nitrification and density of nitrifying microorganisms are affected by substrate storage time and lime and peat amendments. Pine tree substrate was manufactured by hammermilling chips of ≈15-year-old loblolly pine trees (*Pinus taeda* L.) through two screen sizes, 4.76 mm (PTS) and 15.9 mm amended with peat (3PTS:1 peat, v:v, PTSP). Pine tree substrate and PTSP were amended with lime at five rates and a peat–perlite mix (4 peat:1 perlite, v:v, PL) served as a control treatment for a total of 11 treatments. Substrates were prepared, placed in plastic storage bags, and stored on shelves in an open shed in Blacksburg, VA. Subsamples were taken at 1, 42, 84, 168, 270, and 365 days after storage. At each subsampling day, each substrate was placed into 12 1-L containers. Six of the 12 were left fallow and six were planted with 14-day-old marigold (*Tagetes erecta* L. 'Inca Gold') seedlings; all containers were placed on a greenhouse bench. Substrates were also collected for most probable number (MPN) assays for nitrifying microorganism quantification. Substrate solution pH, electrical conductivity (EC), ammonium-N (NH_4-N), and nitrate-N (NO_3-N) were measured on fallow treatments. Marigold substrate solution pH, EC, NH_4-N, and NO_3-N were measured after 3 weeks of marigold growth. Nitrate-N was detected in fallow containers at low concentrations (0.4 to 5.4 mg·L^{-1}) in PTS in all limed treatments at all subsampling days, but in the non-limed treatment, only at Days 270 and 365. Nitrate-N was detected in the fallow containers at low concentrations (0.7 to 13.7 mg·L^{-1}) in PTSP in the 4- and 6-kg·m^{-3} lime rates at all subsampling days. Nitrite-oxidizing microorganisms were present in PTS at all subsampling days with the highest numbers measured at Day 1. Ammonium-to-nitrate ratios for the marigold substrate solution extracts for both PTS and PTSP decreased as pH increased. This study shows that nitrifying microorganisms are present and nitrification occurs in PTS and PTSP and is positively correlated to substrate pH.**

Nitrification, the biological oxidation of reduced forms of nitrogen (N) to nitrate (NO_3^-), affects the fertilizer management of nursery and greenhouse crop production. In general, plants grow best in a combination of NH_4-N and NO_3-N (Barker and Mills, 1980). The extent of nitrification in container substrate will influence fertilizer N choice. If nitrification does occur, less expensive NH_4^+ or urea-based fertilizers can be used. The occurrence of nitrification is also an environmental issue. Anionic NO_3^- is more easily leached from container substrates than NH_4-N forms (Stowe et al., 2010). The occurrence of nitrification impacts the amount of NO_3-N leached from containers, subsequently entering runoff from a production site, and contaminating waterways and groundwater. Furthermore, the production of nitrous and nitric oxide, either as byproducts of NH_4^+ oxidation or as intermediates in the process known as nitrifier denitrification, are gases that add to the greenhouse effect of the earth's atmosphere. Nitrification also acidifies the substrate (soil) and may affect nutrient form and availability and subsequently plant growth.

Autotrophic nitrification, thought to be responsible for the majority of NH_4^+ oxidation in most soils, is carried out by two distinct groups of chemolithotrophic bacteria, bacteria that derive their energy from oxidizing inorganic compounds and fix CO_2 to produce organic carbon. Ammonia-oxidizing bacteria (AOB) oxidize NH_4^+ to nitrite (NO_2^-) while nitrite-oxidizing bacteria oxidize NO_2^- to NO_3^-. Ammonia-oxidizing bacteria grow in a pH range of 5.8 to 8.5 and have growth optima in the range of 7.5 to 8.0 (Prosser, 1989). The generally accepted reason for this sensitivity is that pH determines the proportions of NH_4^+ and NH_3 present. The pKa value of the NH_4^+/NH_3 pair is 9.25; thus, NH_4^+ and NH_3 will be in equal proportions at pH 9.25. There will be more NH_4^+ than NH_3 below pH 9.25 and the converse will occur above pH 9.25. Ammonia (the actual substrate for the oxidizing enzyme) passively diffuses into bacterial cells, but NH_4^+ transport into cells is energy-dependent and, once inside, must be deprotonated for use as substrate (Prosser, 1989).

A wide variety of heterotrophic fungi and bacteria can oxidize NH_3 or reduced N from organic compounds to hydroxylamine, NO_2^-, and NO_3^-. No energy is derived from this conversion and rates are generally much lower than autotrophic nitrification (Prosser, 1989). This heterotrophic pathway is thought to occur in some acid forest soils (Brierley and Wood, 2001; Lang and Jagnow, 1986).

Nitrification has been verified in peat (Elliott, 1986) and pine bark (Niemiera and Wright, 1986b) substrates, two commonly used substrates in the greenhouse and nursery industries. Studies with these substrates have shown nitrification to be sensitive to pH, temperature, and concentration and form of supplied N. Nitrification rate increased with increasing pH (Niemiera and Wright, 1986a; Vetanovetz and Peterson, 1990) and with increasing temperature (Niemiera and Wright, 1987b). However, Walden and Wright (1995) found that temperatures greater than 46 °C had a negative impact on nitrification in a pine bark medium. Nitrification rate increased with increasing NH_4^+ fertilizer concentration in pine bark (Niemiera and Wright, 1987a). In peat-based substrate, nitrification activity was greater when a 1 NH_4-N:3 NO_3-N ratio was used than with either a 1:1 or a 3:1 ratio (Lang and Elliott, 1991).

Preliminary studies (L. Taylor, unpublished data) showed that nitrite-oxidizing microorganisms occur in recently manufactured and aged PTS, a relatively new alternative to pine bark and peat-based substrates (Wright and Browder, 2005; Wright et al., 2008), but nitrification in PTS has not been documented. Pine tree substrate is manufactured from trunks of ≈15-year-old loblolly pine trees (*Pinus taeda* L.) by chipping and hammermilling to a desired particle size. Like with other substrates, PTS is stored by manufacturers and growers for later sale or use. Recently manufactured PTS has a pH value within the recommended range for soilless substrates, 5.4 to 6.5 (Nelson, 2003), but pH decreases with storage time (Taylor et al., 2012). Pine tree substrate is often amended

Received for publication 14 Sept. 2012. Accepted for publication 7 Nov. 2012.
The research was funded in part by the Virginia Agricultural Council and the Virginia Nursery and Landscape Association.
Use of trade names does not imply endorsement of the products named nor criticism of similar ones not mentioned.
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