

INOCULATING



omposted Pine Bark

with beneficial organisms
to make a disease suppressive compost
for container production
in Mexican forest nurseries

José Villa Castillo |

ABSTRACT

At a Mexican nursery, we compost pine bark, inoculate it with beneficial microorganisms, and use it to grow conifer seedlings in containers for reforestation. In this article, I describe how we make composted pine bark (CPB), a technically and economically viable alternative to expensive, imported growth substrates. CPB is easy to produce and can be made on a small scale, generating permanent nursery and reforestation jobs and promoting the stability of Mexican forest plantation companies. We have found that using CPB minimizes use of fungicides in the nursery. I discuss how to inoculate composted bark with bacteria (*Bacillus* spp.) and fungi (*Trichoderma* spp.) to enhance suppression of root disease organisms.

KEY WORDS

inoculation, CPB, *Bacillus subtilis*, *Trichoderma* spp.

NOMENCLATURE

(Mexican pines) Farjon and others (1997); (other plants) USDA NRCS (2004); (fungi) Farr and others (2004)

The Ciudad Guzmán forest nursery is located in Guzmán City, Municipality of Zapotlán el Grande, Jalisco, México. The nursery has an area of 5 ha (12 ac) located at an altitude of 1540 m (5050 ft), under a sub-humid temperate climate with summer rains. Founded in 1954 by Atenquique Industrial Company, the nursery produced forest seedlings using the traditional polybag system (plastic bags filled with native soil) until 1996. In 1997 the nursery was one of the first Mexican nurseries to produce container forest seedlings in a composted pine bark (CPB) growth medium and to introduce an automated fertigation system. This innovation was possible through the Forestry Development Program of the Jalisco State Government in cooperation with Fundación Chile, a Chilean governmental foundation. Our use of CPB and containers resulted in enhanced outplanting performance, a crucial factor considering the urgent necessity to reforest degraded forest lands of México.

Tree bark is a by-product commonly discarded from México's sawmill and cellulose industry. For more than 25 y, tree bark has been the main growth medium commonly used in several countries that lack a considerable source of peat moss. During the

181



Figure 1. Custom built hammer mill.
Photo by José Villa Castillo



Figure 2. Compost piles at Ciudad Guzmán Forest Nursery, Jalisco México.
Photo by José Villa Castillo

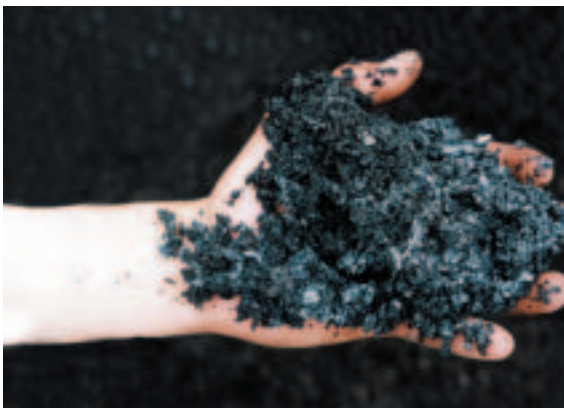


Figure 3. Composted pine bark in the final stages of the process showing the development of actinomycetes and their white-colored mycelium.
Photo by José Villa Castillo

1970s, use of pine bark as a substitute for peat moss boomed in Europe and the US because of an increase in the price of peat moss and restrictions on disposal of bark. Consequently, many industries transformed a waste product into a usable one by using tree bark for a variety of horticultural and ornamental purposes.

The bark of many species, including softwoods (conifers) and hardwoods (broadleaf species), is used. Typical conifers used include various species of pines (*Pinus* L. [Pinaceae]). Typical broadleaf trees used include species of *Populus* L. (Salicaceae), *Quercus* L. (Fagaceae), *Ulmus* L. (Ulmaceae), and *Acer* L. (Aceraceae). Mechanically peeled bark is usually ground with a hammer mill and the eventual by-product may be a combination of various species.

Tree bark often requires composting before using it as a growth medium. Composting is the decomposition of recognizable organic material into unrecognizable organic matter (humus) by the action of a wide spectrum of organisms under optimum conditions of temperature, humidity, and aeration. The principal functions of composting are to eliminate possible phytotoxic components and reduce, through microbial activity, the high carbon-to-nitrogen ratio (C:N) inherent in bark. During decomposition microbes use most of the available N to facilitate their consumption of organic matter as an energy source. Without adequate composting before seedling production, the surge of N use by microorganisms may induce N deficiencies in the crop. Bark decomposes slowly, more so in conifers than in broadleaf species. This characteristic provides structural stability to the growth medium and explains why pine bark is used to produce woody species with extended growing cycles.

BENEFITS OF COMPOSTED PINE BARK

Generally, pine bark has a cation exchange capacity of 40 to 80 meq/l, a pH

that ranges from slightly acid to neutral, low electrical conductivity (0.1 to 0.6 mmhos/cm), and many cracks that allow for water and air storage and movement. Pine bark has a total porosity of around 80% with a high aeration capacity and high retention of water in micropores. The bulk density varies between 100 and 400 kg/m³ (168 to 336 lb/yd³) on a dry weight basis (Burés 1997).

Bark suppresses development of plant pathogens like *Pythium irregulare* Buisman, *Thielaviopsis basicola* (Berk. and Broome) Ferraris and species of *Phytophthora* de Bary, *Rhizoctonia* DC, and *Fusarium* Link: Fr. (Hoitink 1990; Peñuelas and Ocaña 1994; Burés 1997; Boyd-Wilson and Walter 2002). In addition, bark has nematocidal effects against several species of nematodes. These suppressive effects depend on the composting length of time and the origin (species) of the raw material. The mechanisms may be related to enhanced porosity; the presence of antagonistic microorganisms such as species of *Trichoderma* Pers.: Fr., *Penicillium* Link: Fr., *Aspergillus* P. Mich. ex Link: Fr., and *Bacillus*; and/or fungicidal properties.

MAKING COMPOST

The fundamental principles of our composting process were extracted from FAO (1991), but the practical aspects described below were developed or refined at the Ciudad Guzmán Nursery.

We begin by screening bark of *Pinus douglasiana* Mart., *Pinus devoniana* Lindl., and *Pinus pseudostrobus* Lindl. to remove any nonorganic debris. Screened bark is then ground into 1- to 20-mm-size (0.04- to 0.79-in) particles using a custom-built hammer mill (Figure 1). The ideal C:N is 25 to 35. If the C:N is higher than this, composting will take more time. If the C:N is lower, then N dissipates as ammonia. We adjust C:N by adding 2 to 4 kg of urea per m³ (3.4 to 6.7 lb/yd³) of original pine bark. Subsequently, it is important to protect the

compost pile against heavy rains and saturated conditions that increase N losses.

In tropical climates, pine bark is usually drier than in temperate climates and will dry out faster during composting. The biological reactions in the pile slow considerably when moisture content drops below 30%. Similarly, saturated conditions impede the reactions by limiting aeration. The optimum moisture content ranges between 50% and 60%—the composting material should be as moist as a wrung sponge. Loss of water can be high, especially in warm weather with natural aeration, and supplemental water may need to be added. Water can be added anytime, but it should always be added during pile construction and when piles are remixed.

We rely on natural air movement, so we restrict the pile size to a maximum of 1.5 m (5 ft) tall and 4.0 m (13 ft) wide to ensure sufficient oxygen in the lower, central regions of the pile (Figure 2). Poor air exchange slows decomposition and favors anaerobic microorganisms that release unpleasant odors as a by-product of their activity.

As decomposition begins, heat is released by microorganism activity. As the pile warms, microorganisms rapidly multiply as they consume the easily digested sugars, starches, and fats. When the temperature reaches 60 °C (140 °F), fungal activity ceases but decomposition continues with the actinomycetes and spore-forming bacteria (Figure 3). A temperature of 55 to 60 °C (131 to 140 °F) for at least 3 d is necessary to kill most weeds and pathogens. This temperature is easily reached in the core of the pile over a 2-mo period. To ensure that all parts of the pile reach this temperature, periodic mixing of the piles (turnings) is required.

Turning of material—done by hand or machine—improves pile aeration, helps to break apart material, and exposes fresh surfaces for microbial attack. When a compost pile is turned before its maturity, fungi invade from the cooler portions and a rise in temperature will occur in concert with renewed

microorganism activity. Pile agitation ensures that most material will be exposed to the higher temperatures. Too much agitation, however, results in excessive cooling and desiccation of the composting material. The turning process can be expensive in terms of labor or machinery costs, which means the number of turnings is a compromise between the cost and the requirements of the final composted product. Simple composting systems like ours that use natural air flux are turned 2 or 3 times per 10-wk interval.

The time between pile construction and maturity of the composting material depends on aeration and moisture content of the pile, as well as environmental conditions. Under optimum conditions, it takes no more than 8 mo for compost production at Ciudad Guzmán Nursery. At maturity, most available food is consumed, and competition between microorganisms begins. Larger soil animals, especially worms, colonize the material, and decomposition and release of heat are very low. The final composted material contains the most resistant parts of the original pine bark, products of the decomposition process, as well as living and dead microorganisms.

INOCULATING COMPOST WITH BENEFICIAL MICROORGANISMS

We have been experimenting with adding *Bacillus subtilis* and *Trichoderma* spp. to our compost piles to enhance root disease suppression. We isolate *B. subtilis* and *Trichoderma* spp. from the composting pine bark, multiply them in the laboratory, and reapply them to the composting piles or to young nursery container plants. See sidebar on page 184 for protocols.

PLANT PRODUCTION WITHOUT FUNGICIDES

From our 5 y of experience at Ciudad Guzmán Nursery and another year of testing at several Jalisco state nurseries, we feel

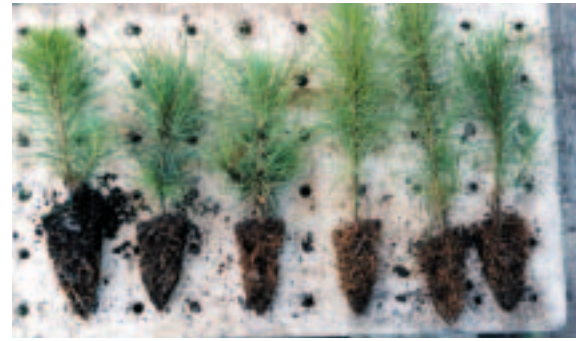


Figure 4. Healthy 8-mo, 20-cm (8-in) pine seedlings grown in CPB, in 195-cm³ (12-in³) containers (60-cell tray). Photo by José Villa Castillo

we have sufficient anecdotal evidence that properly composted and inoculated pine bark can be used as a growth medium to propagate tree species without the use of fungicides (Figure 4). After these nurseries began growing seedlings in CPB, damping-off problems have been eliminated without the need for fungicide.

CONCLUSIONS

The Mexican sawmill industry can reduce waste and environmental pollution by using its pine bark by-products as the substrate for growth media. At Ciudad Guzmán Nursery, conversion of seedling production from traditional polybags filled with native soil to a container system using CPB as growing medium dramatically improved outplanting performance of pines in México. Furthermore, using CPB has allowed us to minimize use of fungicides in the nursery, reducing production costs and chances of groundwater pollution. Using CPB rather than native soil in seedling production supports, rather than contradicts, one of the main objectives of restoration: soil stabilization and prevention of erosion. CPB is a technically and economically viable alternative to expensive, imported substrates for container nurseries, such as peat moss. Reducing costs contributes to the stability of Mexican forestry companies. The CPB industry has created permanent jobs in the nursery and reforestation fields.

Protocols for isolating, multiplying, and reapplying *Bacillus subtilis* and *Trichoderma* spp.

PROTOCOL FOR *BACILLUS SUBTILIS*

Isolation

1. Collect several 10-g (0.35-oz) samples from mature composting pine bark piles. (At Ciudad Guzmán Nursery, this is at 8 mo.)
2. Dilute 1 g (0.035 oz) of compost bark in 100 ml (3.4 fl oz) of distilled and sterilized water.
3. Prepare ADP (agar, dextrose, potato) substrate for Petri dishes:
 - Boil 200 g (7 oz) of sliced and non-peeled potatoes in water for 20 min.
 - Strain out and discard water.
 - Mash potatoes and place in a 1-l beaker.
 - Add 10 g (0.35 oz) of dextrose and 12 g (0.42 oz) of agar. Mix well. Add distilled water for a total volume of 1 l (34 fl oz).
 - Sterilize in an autoclave at 120 lbs for 15 min.
 - Pour into clean Petri dishes and let cool and harden.

4. Collect solution from Step 2 with a flame-sterilized platinum thread inoculating loop (cooled in agar), and spread it over the agar surface in the Petri dishes, one dip per dish.
5. Incubate at room temperature (20 to 25 °C [68 to 77 °F]) for 72 h.
6. Identify *B. subtilis* colonies under the microscope by their distinctive morphological structures (Herrera and Ruiz-Oronoz 1968), remove them from original Petri dishes, and use them to inoculate other Petri dishes containing clean ADP. Repeat steps 5 and 6 until pure *B. subtilis* cultures are obtained.

Multiplication

7. In a pressure cooker combine sliced, non-peeled potatoes and distilled water at a ratio of 200 g (7 oz) of potatoes per liter of water (33 fl oz). Cook for 20 min, then strain out and discard the cooked potatoes. Place the remaining liquid in a galvanized steel container with a lid and add 10 g of table sugar (sucrose) per liter of liquid (0.35 oz/34 fl oz). Do NOT add agar. Cover container and sterilize at 80 °C (176 °F) for 30 min. Cool to room temperature.
8. Remove small pieces of pure *B. subtilis* cultures from petri dishes and add them at a rate of 4 to 6 pieces of culture to 20 l (5.3 gal) of the potato and sugar solution, in the same containers in which the solution was made.
9. Oxygenate medium using an air pump with filter, and incubate medium at room

PROTOCOL FOR *TRICHODERMA* SPP.

Isolation

1. Collect several 10-g (0.35-oz) samples from mature composting pine bark piles. (At Ciudad Guzmán Nursery, this is at 8 mo.)
2. Dilute 1 g (0.035 oz) of composted bark in 100 ml (3.4 fl oz) of distilled and sterilized water.
3. Prepare ADP (agar, dextrose, potato) substrate for Petri dishes:
 - Boil 200 g (7 oz) of sliced and non-peeled potatoes in water for 20 min.
 - Strain out and discard water.
 - Mash potatoes and place them in a 1-l beaker.
 - Add 10 g (0.35 oz) of dextrose and 12 g (0.42 oz) of agar. Mix well. Add distilled water for a total volume of 1 l (34 fl oz).
 - Sterilize in an autoclave at 120 lb for 15 min.
 - Pour into clean Petri dishes and let cool and harden.

4. Collect solution from Step 2 with a flame-sterilized platinum thread inoculating loop (cooled in agar), and spread it over the agar surface of the Petri dishes, 1 dip per dish.
5. Incubate at room temperature (20 to 25 °C [68 to 77 °F]) for 72 h.
6. Identify *Trichoderma* colonies under the microscope by their distinctive morphological structures (Alexander 1980), remove them from original Petri dishes, and use them to inoculate other Petri dishes containing clean ADP. Repeat steps 5 and 6 until pure *Trichoderma* cultures are obtained.

Multiplication

7. Once a pure culture is obtained, transfer a 1-cm² (0.16-in²) growth media section into a glass container holding 400 ml (13.5 fl oz) distilled and sterilized water, 10 g (0.35 oz) of dextrose, and 1 g (0.035 oz) of ammonium sulfate.
8. Place containers in a dark room at 25 °C (77 °F) for 7 d.
9. Shake containers to mix fungi particles with growing media. Inoculate 1 kg (2.2 lb) of composting bark previously sterilized in an autoclave (15 lb for 30 min) with 100 ml (3.4 fl oz) of *Trichoderma* solution. Transfer inoculated bark into a glass container and incubate in a dark room at 20 to 25 °C (68 to 77 °F) for 20 to 30 d, after which *Trichoderma* will have colonized the composted bark flakes producing *Trichoderma* compost.

The translation work of Gabriela Buamscha is most appreciated.

REFERENCES

- Alexander M. 1980. Introducción a la Microbiología del Suelo. AGT editor, México.
- Boyd-Wilson HSH, Walter M. 2002. Suppression of Fusarium seedling blight by composted and uncomposted radiata pine bark. *Australasian Plant Pathology* 31:57–61.
- Burés S. 1997. *Sustratos*. Ed. Agrocencias. Spain. 340 p.
- Farjon A, Perez de la Rosa JA, Styles BA. 1997. A field guide to the pines of Mexico and Central America. United Kingdom: Royal Botanic Garden, Kew. 147 p.
- Farr DF, Rossman AY, Palm ME, McCray EB. 2004. Fungal databases. URL: <http://nt.ars-grin.gov/fungaldatabases> (accessed 13 Sep 2004). USDA Agricultural Research Service, Systematic Botany and Mycological Laboratory.
- [FAO] Food and Agriculture Organization of the United Nations. 1991. Manejo del suelo: producción y uso del composte en ambientes tropicales y subtropicales. Rome, Italy: FAO Boletín de suelos No. 56. 178 p.
- Harvey LM, Smith JE, Kristiansen B, Neill J, Senior E. 1996. The cultivation of ectomycorrhizal fungi. Glasgow, Scotland, United Kingdom: University of Strathclyde.
- Herrera T, Ruiz-Oronoz M. 1968. *Botánica criptogámica*. Eclalsa, México.
- Hoitink HAJ. 1990. Production of disease suppressive compost and container media, and microorganism culture for use therein. US Patent 4960348. 13 Feb 1990.
- Peñuelas R, Ocaña JL. 1994. Cultivo de plantas forestales en contenedor. Ministerio de Agricultura Pesca y Alimentación. Ed. Mudi-Prensa. Spain. 189 p.
- Rose R, Carlson WC, Morgan P. 1996. The target seedling concept. In: Rose R, Campbell SJ, Landis TD, editors. Target seedling symposium: proceedings, combined meeting of the western forest nursery associations; 1990 Aug 13–17; Roseburg, Oregon. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-200. p 1–8.
- USDA NRCS 2004. The PLANTS database, version 3.5. URL: <http://plants.usda.gov> (accessed 14 Sep 2004). Baton Rouge (LA): National Plants Data Center.

AUTHOR INFORMATION

José Villa Castillo
 Technical Advisor
 Santa Ana No 31
 Col Santa Cecilia
 Ciudad Guzmán
 Jalisco, CP 49000
 México
josevc@ipnet.com.mx
oyamel_2001@yahoo.com.mx

temperature for 72 h. Solution is ready when it smells sweet but not strong. Readiness can be confirmed by observing a large population of *B. subtilis* in a drop of solution under the microscope.

Reapplication

10. Reinoculate the composting piles with *B. subtilis* at a ratio of 20 l solution per 20 m³ (4 gal/20 yd³) pile.

Reapplication

Use *Trichoderma* compost in 2 ways:

- a. To reinoculate composting piles: Add 1 kg (2.2 lb) of *Trichoderma* compost to 1000 l water and apply to a 20-m³ composting pile (50 l/m³ [9.6 gal/yd³]).
- b. To inoculate seedlings: Dilute 1 kg of *Trichoderma* compost in 60 l of water (2.2 oz/gal). Filter this solution with a fine mesh kitchen strainer and spray over young seedlings at a rate of 60 l solution/600 m² crop area (0.31 fl oz/ft²). (At Ciudad Guzmán Nursery 600 m² is equivalent to approximately 130 000 seedlings.)