Operational Ectomycorrhizal Fungus Inoculations in Forest Tree Nurseries: 1989

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ABSTRACT. During the past 15 years, the mycorrhizal research and development program has evolved to the practical, efficient, and cost-effective application of the ectomycorrhizal fungus, Pisolithus tinctorius (Pt), in both container and bare-root nurseries. The benefits of Pt in reforestation, mineland reclamation, and Christmas tree production include significant increases in nursery seedling quality (reduced culls), and increased survival and growth in field outplantings. Four types of commercial inoculum are currently available, including vegetative mycelium, bulk spores, spore pellets, and spore-encapsulated seeds. Custom equipment has been developed and is commercially available for the operational application of vegetative inoculum in bare-root nurseries. The demand for custom-grown, Pt-inoculated seedlings for an expanding variety of forest applications continues to increase. Approximately 6.5 million seedlings were inoculated with Pt in 1989 in bare-root and container nurseries in the Southern, Central, and Eastern United States. Technology is being expanded to other ectomycorrhizal fungi, host tree species, forest applications, and geographic locations.

Additional Keywords: Ectomycorrhizae, Pisolithus tinctorius, bare-root nurseries, container nurseries, seedling quality, field forestation, mineland reclamation, Christmas tree production, commercial inoculum types, inoculation techniques.

During the past 15 years, the USDA Forest Service, in cooperation with a number of state and private forestry agencies, has been conducting extensive research on mycorrhizae and their applications in forest tree nurseries, forestation, mineland reclamation, and other related forestry uses such as Christmas tree production. The primary objective of this project has been the practical application of one ectomycorrhizal fungus, Pisolithus tinctorius (Pt), in forest land management. This fungus was selected because of is availability, ease of manipulation, wide geographic and host range, and demonstrated benefits to a wide variety of host trees. Pt is especially tolerant of extreme soil conditions, including low pH, high temperatures, and drought, that frequently either kill or inactivate other less tolerant ectomycorrhizal fungi and their host trees (Marx, Cordell, and others 1984).

During the past 10 years, the Pt ectomycorrhizal research and development program has evolved from the controlled nursery-plot research phase to relatively large-scale operational applications in both bare-root and container seedling nurseries. Ectomycorrhizae fungus technology is rapidly expanding to include additional fungi and tree hosts for a variety of forestation applications throughout the U.S. and in several foreign countries. Operational applications are expanding in the United States (Cordell, Caldwell, and others 1988; Castellano, Trappe, and Molina, 1985; Hung and Molina, 1986; Trappe, 1977), the Philippines (Bartolome, de la Cruz, and others, 1989), France (LeTacon, Garbaye, and others, 1988), and Canada (Langlois and Gagnon, 1988). Effective Pt inoculum, along with the necessary equipment and technology for successful operational applications in bare-root and container nurseries, is now available to nursery personnel. However, the decision to incorporate ectomycorrhizal fungus inoculations into the nursery management program is shared jointly by the nurseryman and the forest land manager. Therefore, nurserymen, contract tree planters, mineland reclamation specialists, Christmas tree growers, and other forest land managers are challenged to become familiar with and evaluate the benefits and costs of custom-grown mycorrhizal-tailored seedlings.

Benefits

Pisolithus tinctorius, along with several other ectomycorrhizal fungi, have provided significant benefits for field forestation, Christmas tree production, and mineland reclamation projects. Numerous conifer and a few hardwood species have been artificially inoculated. National container and bare-root nursery evaluations have demonstrated the effectiveness of different formulations of the Pt inoculum on selected conifer seedling species (Marx, Ruehle, and others 1981; Marx, Cordell and others 1984). During the past 15 years, more than 100 bare-root nursery tests with Pt have been

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conducted in 38 states. Results obtained from 34 nursery tests showed that Pt inoculated southern pine seedlings had a 17 percent increase in fresh weight, a 21 percent increase in ectomycorrhizal development (Fig. 1), and a 27 percent decrease in the percent of cull seedlings at lifting time (Fig. 2). The few instances of negative results have been positively correlated with such factors as ineffective Pt inoculum, adverse environment, detrimental cultural practices, and pesticide toxicity.

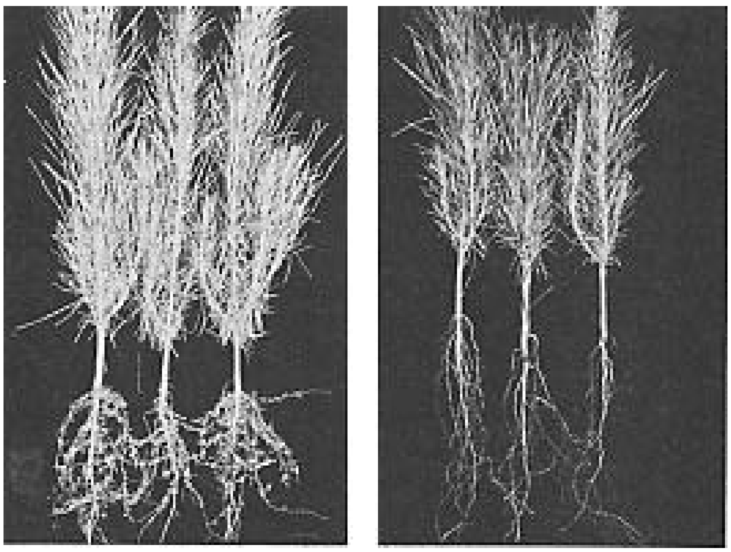

Figure 1. 1-0 loblolly pine seedlings with Pt ectomycorrhizae (Left) and with only naturally occurring ectomycorrhizal fungi (Right)

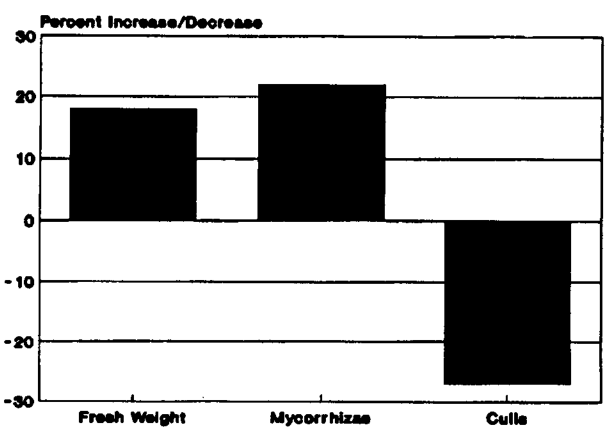

Figure 2. Effects of inoculation with Pt vegetative mycelium on southern pine seedlings in 34 bareroot nurseries.

Inoculated seedlings have been planted on a variety of routine forestation sites, mineland reclamation sites, kaolin wastes, and Christmas tree farms in locations throughout the United States. Over 100 Pt outplantings involving 12 species of conifers are being monitored in 20 states on a variety of forestation, mineland reclamation, and Christmas tree sites. Preliminary analyses show significant increases in tree survival and/or growth in over half of the 100 + field studies. Pt-inoculated loblolly pines Pinus taeda L.) show significant increases in tree volume growth, when compared with uninoculated check trees on routine forestation sites in four Southern States (Fig. 3). Positive field responses have been correlated with successful Pt nursery inoculations (Pt Index $\geq$ 50) and with periodic moisture stress. Results from outplanting studies in southern Georgia show that loblolly pine seedlings with abundant Pt ectomycorrhizae at planting date are more capable of withstanding certain site and/or environmental stresses than seedlings without Pt ectomycorrhizae. Rainfall deficiencies have been frequently associated with large growth differences. Results from two studies (Marx, Cordell and Clark 1988; Marx and Cordell, 1988) on routine forestation sites support the theory of greater drought tolerance of Pt-inoculated seedlings. After 4 years on a good quality, formerly forested site in south Georgia (site index = 80 ft. at age 25), trees with only naturally occurring Thelephora terrestris ectomycorrhizae had significantly less growth during years of low rainfall than Pt-treated trees (Marx, Cordell, and Clark, 1988). During years with high moisture stresses, Pt ectomycorrhizae markedly improved diameter growth. The apparent effectiveness of Pt-inoculated pine seedlings in tolerating moisture stress on routine forestation sites is highly significant and should greatly expand the economic practicality of the Pt program in forest land management.

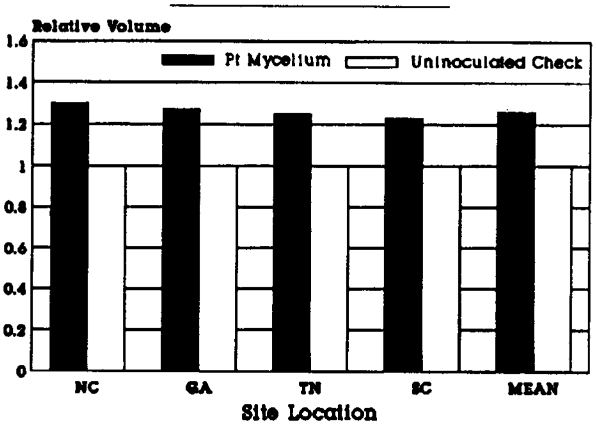

Figure 3. Positive growth responses to Pt inoculation by loblolly pine after 6 to 10 years on routine forestation sites in 4 southern states.

Extensive reclamation research has been conducted on seedlings custom grown with Pt ectomycorrhizae and outplanted on disturbed and adverse sites of various types in the Eastern U.S. In numerous field tests on abandoned mine sites, annual tree root evaluations have confirmed the ecological adaptation of Pt to these adverse sites. Without exception, seedlings with Pt ectomycorrhizae developed new roots very rapidly, and these roots were quickly colonized by the fungus. Root growth was also routinely followed by the prolific production of Pt fruiting bodies in the vicinity of trees with Pt ectomycorrhizae on their root systems. Outplantings established by the Ohio Division of Mineland Reclamation in southern Ohio during the past 7 years continue to show significant tree survival and/or growth increases for Pt inoculated Virginia (P. virginiana Mill.) eastern white (P. strobus L.), and loblolly x pitch (P. rigida Mill.) hybrid pines and northern red oak (Quercus rubra L.) seedlings over routine nursery seedlings (Fig. 4). More recently, Pt-inoculated eastern white and Virginia pines showed increased first-year survival results over uninoculated trees in Christmas tree plantings in North Carolina and Alabama (Fig. 5).

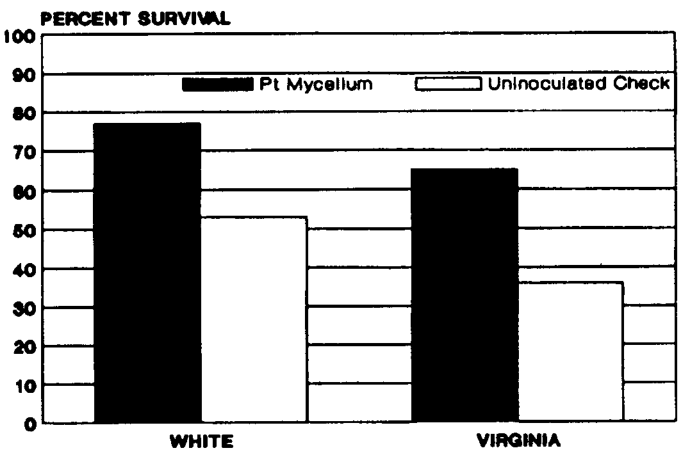

Figure 4. Survival of two pine species on mine reclamation sites in Ohio

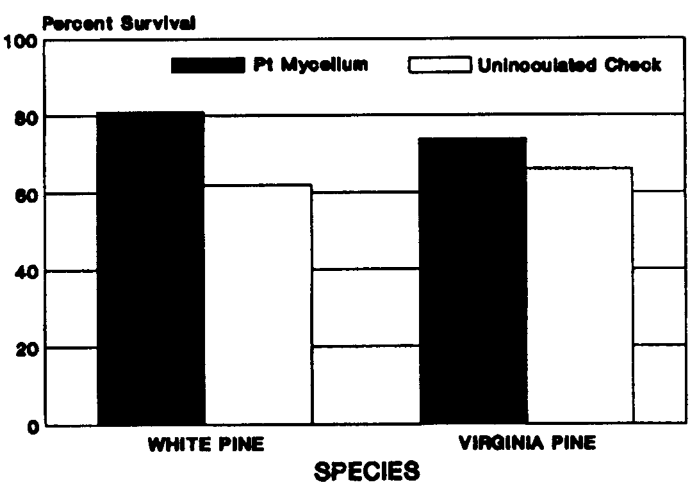

Figure 5. Survival of two pine species in two Christmas tree plantings after one year in the field.

Nursery Inoculations

The technology, commercial fungus inoculum, and inoculation equipment necessary to manage the Pt ectomycorrhizal fungus have been developed and are available to nurserymen for operational use. The types of Pt inoculum that are commercially available include vegetative inoculum from Mycorr Tech, Inc., University of Pittsburgh Applied Research Center, Pittsburgh, Pa.; and spore pellets, spore encapsulated seeds, and bulk spores from either International Tree Seed Co., Odenville, Al., or South Pine, Inc., Birmingham, Al. The ectomycorrhizal fungus inoculum applicator has been redesigned with considerable modifications to facilitate the efficient and effective application of Pt vegetative inoculum prior to sowing in bare-root nurseries. Additional improvements in technology and equipment include the development of a machine for side-banding vegetative inoculum between rows of established seedlings. Both applicators are commercially available from R. A. Whitfield Manufacturing Co., Mableton (Atlanta), Ga.

Nursery inoculation procedures vary, depending upon the type of inoculum used. However, with either vegetative or spore inoculum, the biological requirements of a second living organism are added to those of the seedling. As a result, special considerations and precautions are required for shipping, storage, and handling of the Pt inoculum, as well as controlling certain aspects of seedling production, lifting, handling, and field planting. Detailed procedures for handling and storage of the various Inoculum types, along with alternative inoculation techniques in bare-root and container nurseries, have been presented (Cordell, Marx, and Owen 1986; Cordell, Owen, and Marx 1987). For successful Pt inoculation in bare-root seedbeds, populations of pathogenic, saprophytic, and native ectomycorrhizal fungi that may already be established in the soil must be reduced. Therefore, soil fumigation before seed sowing (preferably in the spring) with effective soil fumigants comparable to the methyl bromide-chloropicrin formulations is required.

Nursery Management Considerations

Guidelines for mycorrhizal nursery management are designed primarily to promote and maintain healthy seedling root systems (Cordell, Owen, and Marx, 1987). One must consider development and retention of seedling feeder roots and mycorrhizae from the time of seed sowing to seedling lifting in the nursery and subsequent tree planting in the field. Nurserymen, field foresters, reclamation specialists, Christmas tree growers, and tree planters must be made aware of the two symbiotic living organisms they are handling – the tree seedling and its complement of mycorrhizal fungi.

Mycorrhizae require generally the same moisture, fertility, and pH as their host tree seedlings, but tolerance for extreme or adverse conditions does vary. Soil and cultural factors that significantly affect mycorrhizae include pH, drainage and moisture, fertility, fumigation, pesticides, cover crops, shading and root pruning. Soil and water pH values are two of the most limiting factors in the development of ectomycorrhizae in both bare-root and container nurseries. In addition, seedling lifting, storage, and planting practices have significant effects on seedling feeder root and ectomycorrhizae retention, quality, and subsequent field survival and growth. Special care must be taken during all stages of seedling handling to maintain sufficient root systems and ectomycorrhizae. Ectomycorrhizae are delicate structures that can be ripped off and left behind in seedling beds during lifting, desiccated in storage, or cut off prior to field planting. To sustain seedling quality, lifting and handling techniques must be modified to minimize damage to feeder roots and ectomycorrhizae. Stripping of roots has severe negative impacts on seedling field performance (Marx and Hatchell 1986). Full bed seedling harvesters are generally less destructive on seedling roots and ectomycorrhizae than single- or double-row lifters. During transfer of the seedlings from the field to the packing room and at all other times when the seedlings are being handled, special care is required to avoid drying of the roots by exposure to wind and sun.

The procedure by which seedlings are packed influences their ability to endure storage and survive field planting. If extended refrigerated storage is required, Kraft paper (KP) bags with a polyethylene seal will maintain seedling moisture better than seedling bales. Cold storage is vital to minimize seedling respiration. Studies comparing packing materials have determined that seedling survival is better when peat moss, clay, or inert water-absorbents are used rather than hydromulch (Cordell, Kais, and others, 1984). Numerous studies have documented the adverse effects of long storage time on seedling quality. With notable exceptions for highly sensitive species such as longleaf pine (P. palustris Mill.), however, most tree species can be safely stored under refrigeration for 2 to 6 weeks.

Improper transportation to the planting site or rough handling during planting can also severely reduce seedling vigor. Tree planters should understand proper planting methods and the need for them. Where possible, seedlings should be transported under refrigeration. Otherwise, they should be covered and stacked with spacers to avoid high temperature buildup inside the seedling containers. Insulated storage boxes and heat reflective "blankets" have also been effectively utilized for seedling protection during shortdistance transport and temporary storage at the planting site. For machine or hand planting, root pruning at the planting site should be avoided because it eliminates carefully nurtured feeder roots and mycorrhizae. High temperatures, wind, and low humidity desiccate and kill feeder roots and mycorrhizae very rapidly. The first priority in planting should always be to maintain seedling viability and vigor. The rate at which acres are planted is of no consequence if the seedlings do not survive.

Costs

There is a wide range in the cost of commercially available Pt inoculum (Table 1). Inoculum costs of other ectomycorrhizal fungus species, when available, are comparable to Pt. Some nurseries purchase the inoculum and add its cost to the seedling price, while other nurseries prefer that the buyer purchase the inoculum. The Pt vegetative inoculum is sold on a volume basis, while the spore inocula are all sold by weight. The cost of the most expensive vegetative mycelium inoculum ($7.50/1,000 seedlings) represents less than 5 percent of the total plantation establishment costs.

TABLE 1. COMMERCIAL Pt INOCULUM COSTS 1989.

Pt Inoculum Type	Inoculum Costs Per [1] 1,000 Seedlings	Hectare	Acre
Vegetative Mycellum	$ 7.50	$ 13.45	$ 5.45
Spore Encapsulated Seed	2.22	3.98	1.61
Spore Pellets	2.75	4.93	2.00
Double-Sited Bulk Spores [2]	0.43	0.77	0.31

1 - COSTS ARE FOR L0BLOLLY, EASTERN WHITE, AND VIRGINIA PINE BARE-ROOT NURSERIES (269 SEEDLINGS/SQ M. – 25 SEEDLINOS/SQ FT.) & FORESTATION PLANTINGS (1.8 x 3.0 M - 6 x 10 FT. SPACING; 1794 TREES/HA. - 726 TREES/AC.) IN THE SOUTHERN AND EASTERN US.

2 - DOUBLE SIFTING IS REQUIRED FOR EVEN FLOW THROUGH SPRAY NOZZLES. STANDARD SPORES ARE ONLY SIFTED ONCE.

Operational Applications

The demand for Pt-inoculated seedlings continues to increase. In 1988, 6 million seedlings at 12 bare-root and container nurseries in the Southern, Central, and Northeastern United States were inoculated with Pt. In 1989, the total rose to 6.5 million seedlings of eight conifer and one hardwood species (Fig.7).Also, several additional ectomycorrhizal fungus species were commercially produced and operationally utilized in 1989. Annually, since 1987, 1.5 million loblolly and 0.5 to 0.75 million longleaf pine seedlings have been successfully inoculated with Pt and custom grown at the Taylor State Nursery, Trenton, S.C., for forestation plantings at the Savannah

River Forest Station, Aiken, S.C. During each of the 1987-88 and 1988-89 planting seasons, four field demonstration plantings were established comparing various nursery and field treatments, including nursery seedling quality, Pt inoculation, pine species, tree spacing and site preparation. Field measurements of these comparative plantings show significant tree survival increases on Pt-inoculated vs. uninoculated pines. This operational project utilizes 3,500 liters of Pt vegetative inoculum in 35,000 linear feet (6.75 miles) of nursery seedbed annually and is apparently the largest single artificial ectomycorrhizal bare-root nursery Inoculation project to date.

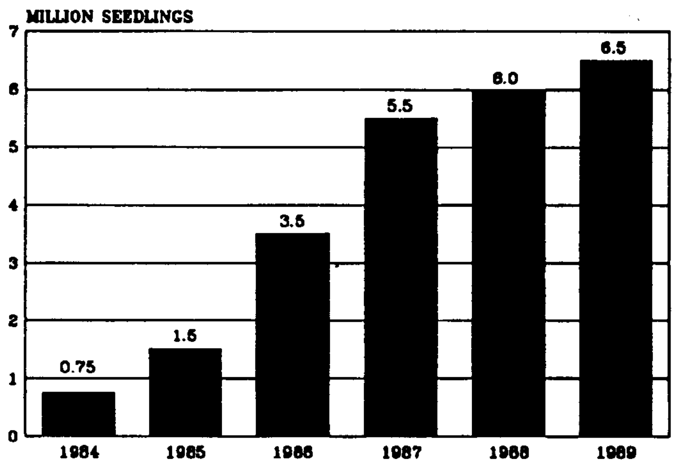

Figure 6. Operational Pt custom seedling production using commercial vegetative and spore inoculum in bare-root and container seedling nurseries, 1984-1989.

Interest in the use of Pt ectomycorrhizae in mineland reclamation has also increased steadily over the past 10 years. Since is inception in 1981, the Ohio Abandoned Mineland Reforestation Program has planted approximately 1.4 million Pt-inoculated seedlings on 810 acres of abandoned minelands in southern Ohio. This program has expanded annually, and in 1988-89 the Ohio Division of Reclamation planted approximately 0.5 million Pt-inoculated seedlings on 285 acres. Estimates for tree planting In Ohio through 1990 indicate further increases of an additional 2.6 million Pt-inoculated seedlings for plantings on 1,850 acres of abandoned mineland (Cordell, Caldwell, and others 1988).

National Forests, state forestry agencies, and a number of private companies have shown considerable interest In the use of Pt ectomycorrhizae on selected forestation sites in the United States. National Forests in Ohio and South Carolina have scheduled the annual production of Pt-tailored bare-root seedlings for selected forestation and reclamation sites. The Savannah River Forest Station, in cooperation with the U.S. Department of Energy (DOE) in South Carolina, has initiated a 5-year reforestation plan utilizing a minimum of 2.0 million Pt-tailored longleaf and loblolly pines annually. During 1989, Pt-inoculated seedlings are being produced for five state forest agencies and five forest products companies. Christmas tree growers have recently ordered Pt-inoculated seedlings, which are presently being produced in several southern nurseries. The demand for Pt-tailored seedlings is expected to substantially increase during the next 5 years due to the increased emphasis on forestation, mineland reclamation and other related forest projects.

Technology Transfer

In a special program, the USDA Forest Service continues to provide mycorrhizae technology to forest tree nurserymen, field foresters, mineland reclamation specialists, Christmas tree growers, and other concerned land managers throughout the United States and several foreign countries (Cordell and Webb 1980; Cordell 1985; Cordell, Owen, and others 1987). Initially, this program emphasized the use of Pt on selected forestation sites and in mineland reclamation programs. However, as previously related, the program is being expanded to a wider range of forestation sites, mycorrhizal fungi, and tree hosts over a broader geographic area. The expanded technology transfer program Is acquiring International recognition.

Conclusions

Results obtained during the past several years consistently demonstrate that the Pt ectomycorrhizal fungus can be used operationally in container and bare-root nurseries to significantly improve survival and growth of seedlings for forestation, mineland reclamation, and Christmas tree production. Technology obtained from this pioneering project is being expanded to other ectomycorrhizal fungi, host tree species, forest applications, and geographic locations. Several types of effective Pt inoculum are commercially available, as are machines for vegetative mycelium Inoculations in bare-root nurseries. These recent developments provide nurserymen, foresters, mineland reclamation specialists, Christmas tree growers, and other land managers with alternatives for using Pt, as well as other selected ectomycorrhizal fungi. The best field planting results continue to be obtained on adverse sites such as coal spoils and forestation sites with soil moisture deficits. In addition, results are consistently better when planted seedlings have Pt indices $\geq$ 50 (Pt incidence equal to or greater than other natural ectomycorrhizae Incidence on seedling feeder roots). The cost of custom seedling inoculations with selected ectomycorrhizal fungi represents only a minor portion of the total tree planting expense (less than 5%), and high seedling quality is an obvious key to successful forestation, mineland reclamation and Christmas tree production. Consequently, the benefits of producing custom-grown seedlings with selected ectomycorrhizal fungi for specific forestation and mineland reclamation sites should greatly exceed the costs.

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