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Project No. 92-T19B - Studies on Control of Armillaria Root Rot and Other Wood Decay Disorders of Almond

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SUMMARY

Armillaria root rot is a lethal disease of *Prunus* and other tree species. In the central valley of California, the disease is caused by *Armillaria mellea* (Vahl.:Fr.) Kummer as determined by us in cooperative studies with Dr. R. Blanchette at the University of Minnesota and Dr. H. Burdsall, USDA, Forest Products Laboratory, Wisconsin. The disease has been controlled with the use of soil fumigants such as methyl bromide or carbon disulfide (gas) and with the use of resistant plum rootstocks such as Mariana 2624 or Myrobalan 29-C. Recently, with the planned cancellation of methyl bromide, we have evaluated a new formulation of carbon disulfide called tetrathiocarbonate (TTC) or Enzone® (Unocal Corp.) and other fumigants, as well as systemic fungicides such as propiconazole (Alamo 1.1EC®, Orbit 3.6EC®) for control of this disease and other wood decay fungi. Long term goals should include studies to determine mechanisms of host resistance in plum rootstocks and to develop breeding programs to select new rootstocks resistant to Armillaria root rot.

In an almond orchard with numerous Armillaria root rot infection centers, we established fumigation sites for individual, replanted trees. For this, diseased trees were removed and the soil was fumigated with one of the following treatments: 1) Preplant methyl bromide; 2) Preplant TTC (3850 µg/ml); 3) Pre- and postplant TTC; 4) Postplant TTC (500 µg/ml); 5) Preplant TTC/*Trichoderma* and postplant TTC/*Trichoderma*; and 6) Non-treated check. The orchard was replanted with almond on the susceptible peach rootstock Lovell. Tree survival was significantly reduced in only the postplant treatments of TTC due to phytotoxicity of the fumigant; whereas other treatments of TTC or methyl bromide were not phytotoxic. As expected, Armillaria root rot requires several years to develop. Trees are continuing to be monitored for the incidence of disease, as well as growth and development of the trees. Additionally, infected root segments were buried at two depths (1 and 4 ft) prior to the soil fumigation treatment and evaluated for the viability of *A. mellea* after 5 and 11 months. In a second orchard in 1994, we repeated the 'buried infected-root' study. None of the fumigation treatments eradicated the fungus from the buried infected root segments after 6 or 12 months. A significant reduction in the recovery of the fungus from buried root segments at 48 inches occurred with the use of methyl bromide, preplant TTC, and pre-/postplant combination treatments of TTC as compared to the check.

In other studies, the effect of fumigants to eradicate the fungus from infected trees by direct application of the chemicals to tree stumps was also evaluated. For this, infected trees that died from Armillaria root rot were cut down, holes were drilled into the stumps to function as reservoirs of the fumigants, and the holes were filled with either metam sodium (Vapam), TTC-liquid formulation, TTC-gel formulation, or they were left untreated as check treatments. The cut end of the stumps were then sealed with silicone sealant and covered with plastic bags that were taped to the trunks with the ends of the bags covered with soil. After 1 year, the fungus was recovered from roots of all of the treatments and thus, no differences were observed between fumigation treatments and the nontreated check. Although this method of fumigant application has been reported

for treatment of conifers with Armillaria root rot in conifer forest ecosystems, this method did not reduce the recovery of *A. mellea* from infected almond trees.

Registration of TTC for control of Armillaria root rot may provide an effective alternative management practice to the use of methyl bromide. Potentially, with the use of resistant rootstocks, pre- and postplant fumigation with fumigants such as TTC, and with systemic fungicides, growers may be able to replant into Armillaria infection centers with minimal risk, and thus, effectively manage the disease on almond. A Section-18 has been approved for pre- and postplant use of TTC on grapevines and the material should be registered for tree fruit crops by the end of 1995 or the beginning of 1996.

In laboratory studies, propiconazole at a concentration of 0.15 μg/ml (ED_{50}) was shown to be effective in inhibiting mycelial growth of *A. mellea*. At concentrations of 10 μg/ml the fungicide was completely inhibitory. Based on these studies, the fungicide was evaluated as a therapeutic treatment for almond trees infected with Armillaria root rot. In 1992 and 1993, trees passively injected with the fungicide were kept alive for two years, whereas trees in similar stages of decline died within 4 months in each year of the evaluation. Non-injurious foliar and trunk applications of potted almond trees, inoculated with the fungal pathogen, are currently being evaluated in greenhouse tests. Potentially, fumigation and systemic fungicides could be utilized as integrated management strategies. Pre- and postplant fumigation treatments could be used to eliminate or reduce inoculum in the root zone of planting sites. Subsequently, as trees grow, applications of a systemic fungicide could be used to prevent infection of healthy trees as their roots grow beyond fumigated zones of soil and contacted new sources of inoculum.

In evaluations of systemic fungicides for control of wood decay organisms in pruning wounds, propiconazole, imazilil, Nectec®, and triadimefon were either applied before or after inoculation of stub cut pruning wounds with the white rot fungus, *Trametes versicolor*. These experiments were initiated in the fall season when pruning of almond is commonly done. In wounds treated and then inoculated, no decay developed in any of the treatments including the non-treated check. In all of these treatments, natural drying and wound healing prevented the establishment of decay. In wounds inoculated first and then treated, decay was most severe in treatments that used sealant materials (e.g. Nectec®). These treatments prevented drying of the wounds and had the smallest zones of discoloration (a host reaction to injury). Currently, both experiments are being repeated. Thus, the use of fungicides or fungicidal sealants to treat tree wounds is questionable under dry conditions where rapid wound healing occurs during fall pruning of almond trees in California.

INTRODUCTION

Armillaria root rot occurs on a wide range of hosts including *Prunus* spp. and other species of tree fruit and nut crops (Raabe 1962, 1979a). Symptoms of the disease are poor shoot growth, premature yellowing and dropping of leaves, dieback of branches, and death of trees. Three signs of the fungus include: 1) white, fan-shaped, mycelial plaques that are formed between the bark and wood; 2) dark brown, root-like mycelial strands called rhizomorphs that are associated with plant roots; and 3) mushrooms or basidiocarps of the fungus, that commonly occur in clusters around the base of tree trunks, are usually produced between October and February. In California, the disease is caused by the putative species *Armillaria mellea* (Vahl.: Fr.) Kummer. Other names of the disease include "Shoestring root rot" and "Oak root fungus" (Ogawa and English 1991).

The fungus attacks roots of plants, killing the cambium and degrading woody tissue. The fungus spreads root to root by rhizomorphs or by mycelium through root contact. Rhizomorphs are the principal agents of infection and can penetrate directly through the bark of roots (Thomas 1934). Under California conditions, basidiospores produced from basidiocarps of the fungus are not considered to function in the epidemiology of the disease. The fungus causes a white rot of wood, degrading all major components of wood cell walls including cellulose, hemicellulose and lignin and survives on dead roots remaining in the soil after tree removal (Ogawa and English 1991).

Soil fumigation and resistant rootstocks of tree crops are the most effective control practices for Armillaria root rot. Resistant rootstocks offer a practical method of control (Raabe 1979b), but trees are generally slower growing and dwarfed. Fumigants reduce inoculum of the fungus but eradication is rarely achieved. Fumigants currently available include metam-sodium (vapam), chloropicrin, methyl bromide, and carbon disulfide (LaRue et al. 1962; Munnecke et al. 1970, 1973, 1981; Filip and Roth 1977). Application methods previously used include treatment of soil (Munnecke et al. 1973) or treatment of stumps (Filip and Roth 1977). Control of Armillaria root rot by soil fumigation is also related to weakening of the fungus followed by antagonism by the fungus *Trichoderma viride* or other *Trichoderma* species (Munnecke et al. 1973; Ohr et al. 1973).

Due to environmental concerns about some fumigants such as methyl bromide, alternative environmentally safe materials need to be evaluated for the control of Armillaria root rot in California. Enzone, manufactured by the Unocal Corporation Chemical Division, is a non-explosive liquid formulation of tetrathiocarbonate (TTC) that releases the fumigant CS_2. Based on information provided by Unocal, Enzone can be used at high concentrations as a pre-plant fumigant or at low concentrations as a post-plant treatment possibly without causing phytotoxicity to almond trees.

Another alternative to the use of fumigants or a treatment to be used in combination with fumigants would be systemic fungicides. Currently there are no fungicides that have proven to be useful in the control of Armillaria root rot. Propiconazole, Alamo 1.1EC, is a systemic fungicide that has be used to successfully control *Ceratocystis* wilt of trees. Although this fungus does not cause root rot like *Armillaria mellea*, it colonizes the vascular system and is difficult to control without the use of systemic fungicides. Potentially, propiconazole may be useful as a control strategy against *Armillaria mellea* and other wood decay fungi.

OBJECTIVES

1) To continue to evaluate replanted trees for Armillaria root rot in fumigation sites established in a commercial orchard.
2) To repeat field studies on the effect of fumigants on *Armillaria mellea* in buried root segments.
3) To determine the species of *Armillaria* in California almond orchards.
4) To evaluate systemic fungicides as a control strategy for *A. mellea* and other wood decay fungi.

MATERIALS AND METHODS

Site selection and isolation of *Armillaria mellea*. A test plot was established in a commercial orchard in Butte County where 10% of a 50 acre block of trees had *Armillaria* root rot. Experiments were done in *Armillaria* infection centers directly affecting approximately 5 acres. One-hundred and eight trees were removed in the fall prior to implementing experimental treatments. Trees that died in the current or previous growing season were identified. Roots of removed trees were sampled and isolations for *A. mellea* were done in the laboratory. For this, root segments were broken open and wood was placed on a selective medium containing 10 mg/ml benomyl (DuPont Co., Wilmington, DE), 100 mg/ml streptomycin, and 2 ml/L of 25% lactic acid. The fungus was identified following cultural characteristics described by Nobles (1965).

Species identification for the putative species *Armillaria mellea*. Both mating-type and vegetative incompatibility studies were conducted to determine the species of *Armillaria*. For this, fruiting bodies of the fungus were collected from an infection center, single-spore isolates were retrieved, and pairings of isolates were done. Additionally, mating tests were done with tester strains to confirm the species of *Armillaria* based on current taxonomic understandings. This research was done in cooperation with the University of Minnesota and the USDA, Forest Products Laboratory in Madison, WI.

Soil fumigation treatments and application techniques. A liquid formulation of Enzone (31.4% TTC; TTC-L) was used for soil treatments. After dead infested trees were removed, soil treatments were: pre-plant TTC-L at 3850 mg a.i./ml (189 L/site); post-plant TTC-L at 500 *u*g/ml (189 L/site); pre- and post-plant

TTC-L; pre- and post-plant TTC-L and root and soil treatments with *Trichoderma harzianum* (see below for details); pre-plant methyl bromide (454 g/site); and nontreated, water drenches as check treatments. Pre-plant treatments were made in December, whereas post-plant treatments (3 months after planting) were made the following May.

Methyl bromide (Dowfume 98%, methyl bromide) was applied by a commercial applicator at each site using a 45 cm soil injector. No coverings (e.g., plastic tarp) were used over the treated soil surface. For applications of TTC-L, a 2 x 3 x 0.5 m basin was prepared at each replant site. Over the next two days, 2 in. (5 cm) of water were applied using sprinkler irrigation or from rain to settle the soil in each basin. At the time of treatment (2 days after irrigation), Unocal representatives observed that each site needed a supplemental application of water. Therefore, another 378 L (100 gal) of water was applied to each site before treatment with TTC-L. An aqueous solution of the fumigant (3850 mg a.i./ml) was prepared and 189 L (50 gal) was applied to each site. Water (189 L) was applied to non-treated control sites. Each treatment had 18 replications (17 replications for the check).

Five days after treatment, soil probes were taken to determine soil moisture at different depths and distances from the center of the prepared basins. Soil probes were obtained from two orchard sites, while 4-8 soil probes were obtained from each of eight treatment sites.

Evaluation of *A. mellea* survival in buried, infested root segments. Approximately, 200 root segments (30 cm long), infested with *A. mellea*, were obtained from removed trees. The segments were stored at 1-3 C for approximately 3 weeks after they were obtained. Isolations were made as previously described to verify the presence of the fungus. Root segments were cut approximately 15-18 cm long and were placed into large (1.25-2.5 cm diam.; 2.0 cm mean) and small diameter (0.5-1.25 cm diam.; 0.9 cm mean) groups. Using steel wire, two root segments (one large and one small diameter segment) were tied together. One pair of segments was tied at one end of a 2.75 m (9 ft) length of steel wire. Another pair of root segments were tied onto the steel wire 91 cm from the first pair. Two holes at treatment sites were dug using a tractor-mounted soil auger. Root segments, attached to the wire, were hung down into each hole at 30 cm (1 ft) and 120 cm (4 ft) depths from the soil line, and the holes were re-filled with soil. The end of each wire was tied to an iron stake. Roots segments were buried in 6 of 17 replications of each treatment prior to the pre-plant treatments. One set of buried root segments from each treatment was removed 5 months, while the second set was removed 12 months after the pre-plant treatments. Data were analyzed using analysis of variance and Least Significant Difference (LSD) mean separation procedures of SAS 6.04.

A second site was also established at the Department of Plant Pathology Field Station, on the campus of University of California, Davis. Roots that were infested with the fungus were collected from trees removed in the commercial orchard described previously. Roots were cut up into small and large segments, the fungus was isolated from each root segments, and two segments were tied together using steel wire and buried in the soil at 30 cm (1 ft) and 120 cm (4 ft) depths as described previously. Sites were treated with fumigants as described above and are currently in the process of being removed and evaluated for the recovery of *Armillaria* from each root segment. For this six replications of each treatment were used.

Replanting of almond trees and *Trichoderma* treatments. In February, one week before planting, basins used for the Enzone drench treatment, were leveled and allowed to settle. Almond trees (Peerless, Carmel, and Butte) on Lovell peach rootstock were replanted into the soil treated sites. Treated soil from the edges of the basin was used to refill the treated area.

During isolation of *A. mellea* from infected root segments, isolates of *Trichoderma harzianum* were obtained and identified. Two isolates of the fungus were sent to Unocal for culturing of the fungus and later use in selected treatments at planting or for post-plant treatments. Dilutions of the batch culture were applied as a root dip or directly to soil with a soil fumigation injector. For this, the isolate JEA S1-16 was grown on a molasses and yeast extract liquid medium (500 ml) on a shaking incubator (25 C) for 14 days, filtered through cheese cloth, and re-suspended in an aqueous suspension of 2% carboxy-methyl cellulose gel. The final

concentration was 5.85 x 10^5 cfu of *T. harzianum* (mycelium and chlamydospores). Roots of 18 trees were dipped into the suspension (0.55 L/tree) and then trees were planted into sites pre-treated with TTC as previously described. Additionally, an equal volume was injected into the soil within a 30 cm radius of the crown of the replanted tree at the time of planting and 3 months later after the post-plant fumigation treatment with TTC (500 mg/ml).

In plot one, at four and nine months after planting, soil samples were collected to a depth of 30 cm using a soil probe (2 cm diam.) from: pre-plant TTC; pre- and post-plant TTC with *T. harzianum* added; methyl bromide; and check treatments. Eight equally spaced soil cores taken from approximately a 30 cm radius of the tree crown were combined in polyethylene bags for a composite sample from each of 10 replications of the four treatments. Soil texture and pH were recorded for the replications of each treatment following methods in Chapman and Pratt (1961). Ten grams, fresh weight of soil for each replication was weighed out and mixed with 100 ml of a 0.1% solution of water agar. A 1/2000 and a 1/10000 dilution were made and 1 ml per plate of each dilution was placed in six petri dishes. A *Trichoderma* selective medium (10 ml/plate) was used in this experiment (Elad at al. 1981). The medium consisted of $MgSO_4$ (0.2 g/L), K_2HPO_4 (0.9 g/L), KCL (0.15 g/L), NH_4NO_3 (1 g/L), glucose (3 g/L), chloramphenicol (0.25 g/L), Lesan 75WP (0.257 g/L), PCNB (0.2 g/L), rose bengal (0.15 g/L), and agar (20 g/L). Plates were incubated at 25 C and numbers of colonies of *Trichoderma* spp. and other fungal species were recorded after 7-10 days. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

Determination of tree survival and decline of replanted trees. Ten and twenty after the pre-plant treatments with fumigants, trees were evaluated for survival, trunk diameter, and decline (0 = healthy; 1 = chlorotic leaves; 2 = chlorotic leaves and thin canopy (no new growth); 3 = chlorosis, thin canopy, and dieback; or 4 = tree death) were evaluated. Data were analyzed using general linear model and LSD mean separation procedures of SAS 6.04.

Suppression/eradication treatments using Enzone. Living, mature trees with Armillaria root rot that bordered infection centers were used in this experiment. The fungus was isolated from wood samples obtained from infected trees as previous described. In May, a soil drench was made using TTC-L (500 *u*g a.i./ml; 189 l per site) applied in basins (2 x 3 x 0.5 m) made around ten infected trees. Water was applied to basins around 10 check trees. Trees were rated using a disease severity scale as described above. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

Direct treatment of infested tree stumps with fumigants. In January 1993, trees that died in the 1992 growing season were cut down leaving a stump approximately 15 cm in height from the ground. Stump diameter was recorded. Seven to eight equally spaced holes (1.9 cm in diam. x 10 cm deep) were made 2.5-3 cm from the perimeter of the trunk using an electric drill. The holes were filled with stock material of either: TTC-L; an Enzone gel formulation (31.4% TTC; TTC-G); vapam (32.7% sodium N-methyldithiocarbamate or Metam-Sodium); or water. The fumigants were poured into each of the stumps (300 ml/stump), bark-corks were placed in each hole, and exposed wood of the stumps was sealed with a silicone tile sealer. After the silicone sealer dried, polyethylene plastic bags were placed over the stumps, the bag edges were buried, and strapping tape was wrapped around the bags and trunks. After 12 months, stumps were removed, roots were sampled, and isolation of *A. mellea* was conducted as previously described to determine if the fungus was eradicated from the infested root system. For each stump, samples were made from primary and secondary roots, as well as from the crown of the tree.

***In vitro* Evaluations of Alamo.** Trees decayed by *Armillaria mellea* were removed from three sites within one orchard in Chico, CA. Decayed roots were removed from each tree and isolations for *A. mellea* were conducted using a Basidiomycete selective medium containing 10 *u*g/ml benomyl and acidified to pH 4 using lactic acid. Cultures were maintained on potato dextrose agar (PDA) medium. To evaluate the fungitoxicity of propiconazole, PDA was made containing 1.0, 0.1, 0.01, 0.001, or 0.00 *u*g/ml (ppm) of propiconazole formulated as Alamo 1.1 EC (Supplied by Ciba Geigy). Plugs of two-week-old cultures of three isolates of *A. mellea* were transferred to fungicide amended media. Each plate was sealed with parafilm and

incubated at 25 C in the light. Each treatment was replicated four times and measurements of colony diameter (mm) were made weekly for four weeks. Values were corrected for plug diameter. Experiments were repeated twice for each isolate of the fungus.

In Situ Evaluations of Alamo. Ten, 8-10 inch diameter almond trees, in a commercial orchard, confirmed to be infected with *Armillaria* were used in this study and rated for their state of decline. Decline ratings were based on the scale: 0 = healthy; 1 = 25% dieback; 2 = 50% dieback and yellowing of leaves; 3 = 75% dieback and some scaffold limbs dead; and 4 = tree death. Photographs of each tree were taken to document severity evaluations. In June 1992, 5 trees were treated with Alamo 1.1 EC at a concentration of 440 ppm using a passive infiltration system and five non-treated trees served as controls. Therapeutic rates were calculated based on Ciba-Geigy Protocol for Alamo use for Dutch Elm Disease Control and volumes were calculated based on 0.5 l per inch diameter tree. For Alamo treatments, 6 medical IV-bags containing 1500 ml each of the Alamo dilution were hung on scaffold branches around the trunk of the tree. Six, 3/8 inch holes were made 3/4 inch deep, two inches below the graft line at 5-6 inch centers around the perimeter of the trunk using a cordless electric drill. Drill bits were disinfected using 400 ppm chlorine solution after each tree. Plastic tygon hose connectors were inserted into the holes and tygon tubes from IV-bags were connected. After 24-hr bags were removed and corks were inserted into drill holes. If bags were not empty, new drill holes were made and IV-bags were re-connected and allowed to drain, as described previously. Trees were evaluated periodically until September 1992. In 1993, the same trees were rated, treated again in June, paired with non-treated trees, and evaluated after 5 months. Foliar applications and preharvest soil drenches of propiconazole (Orbit 3.6EC, Alamo 1.1EC) are currently being evaluated as a non-injurious application method for preventative control and as a possible compliment for fumigants for control of this disease.

Evaluation of pruning wound protection with systemic fungicides. In evaluations of systemic fungicides for control of wood decay organisms in pruning wounds, propiconazole, imazilil, Nectec® (a latex paint containing propiconazole and imazilil) with and triadimefon were either applied before or after inoculation of stub cut pruning wounds with the white rot fungus, *Trametes versicolor*. For this, 0.5 x 1.0 cm holes were drilled into the cut ends of branches approximately 2.0 cm in diameter. Wooden plugs made from 0.5 cm diam. x 0.5 cm wooden dowels that were infested with the fungus were placed in each hole and inserted with a second non-infested plug. Treatments were either applied before or after inoculation. When applied before inoculation, treatments were allowed to air dry for 2 hours. These experiments were initiated in the fall season when pruning of almond is commonly done. After 1 year, inoculated branches were removed, cut longitudinally in half, and the reaction zone and the white rot wood decay was measured from the point of inoculation. For this experiment, six single tree replications were used for each treatment. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

RESULTS AND DISCUSSION

Identification of *Armillaria* species. Mating-type and vegetative incompatibility studies demonstrated that the fungus causing Armillaria root rot of almond in California is *Armillaria mellea*. This research was done in cooperation with the University of Minnesota and the USDA, Forest Products Laboratory in Madison, WI.

Soil characterization and movement of TTC-L in soil. In the commercial orchard, two main soil types, a heavy clay loam and a sandy loam, characterized the upper one meter of the soil profile of fumigation treatments sampled. Below one meter, soils were generally sandy to gravely. Soil pH ranged from 6.32 to 6.58 for all the soil fumigation sites. In the research plot, the soil type was consistently a clay loam throughout the 4 foot soil profile. Soil pH ranged from 6.8-7.2. Movement of TTC from soil basins through the soil profile was estimated by taking soil probes at various distances from basin centers and comparing them to probes taken where no treatments were applied. Lateral movement in heavy soils was less than 2 m, while movement in sandy soils was estimated to be between 3-4 m.

Survival of *A. mellea* in buried root segments. Percent recovery of *A. mellea* after 5 months from infested buried root segments was variable for both depth (30 and 121 cm) roots were buried and for the

treatments evaluated. The two root sizes (0.9 and 2.0 cm) used in the experiment did not significantly influence efficacy of the treatments evaluated. A significant interaction, however, was observed between depth of the root segment and treatment ($P < 0.02$). Thus, the two depths were evaluated separately. For infested roots buried at a depth of 30 cm, percent recovery of the fungus from pre-plant TTC-L and pre-plant TTC-L + *T. harzianum* was significantly reduced from the check; whereas the methyl bromide treatment was intermediate between the TTC-L and check treatments. At the 121 cm depth, percent recovery was significantly reduced for methyl bromide and pre-plant TTC-L treatments when compared to the check and the pre-plant TTC-L + *T. harzianum*. No difference was found between the check and the pre-plant TTC-L + *T. harzianum*. The inconsistency of methyl bromide at the 30 cm depth was possibly due to the application technique (soil injection and no covering over treated soil), whereas the variability of the pre-plant TTC-L at 121 cm may indicate the difficulties inherent in attempting to eradicate *A. mellea* from deeply buried roots.

Table 1. Recovery of *Armillaria mellea* from root segments one year after soil fumigation with methyl bromide or tetrathiocarbonate (TTC).

	Percent Recovery of *A. mellea* from Roots at Two Soil Depths	
Treatment	30 cm	121 cm
Check	66.7 ab	100.0 a
Postplant TTC	83.3 a	100.0 a
Preplant TTC	40.0 ab	50.0 bc
Preplant TTC+*Trichoderma*	80.0 ab	66.0 ab
Pre-+Postplant TTC	20.0 b	25.0 c
Methyl Bromide	42.3 ab	25.0 c

[1] - Root segments were infested with *A. mellea* were buried at one and four foot depths and the sites were treated with fumigants. Roots of replants in the TTC+*Trichoderma* treatment were dipped in a cellulose-gel suspension of *Trichoderma harzianum* prior to planting.

After 1 year, at both soil depths, percent recovery of *A. mellea* was significantly reduced for the pre- and post-plant TTC-L treatment when compared to the check (Table 1). At the 30 cm depth, recovery of the fungus from root segments in the other treatments was not significantly different from the check, however, a general reduction was observed. At the 121 cm depth, a 100% recovery of the fungus was obtained from the check and post-plant TTC-L. Recoveries of *A. mellea* from roots in the methyl bromide, pre-plant TTC-L, or the pre- and post-plant TTC-L treatments were significantly less than the check and post-plant TTC-L treatments. Recovery of *Armillaria* from the pre-plant TTC-L + *T. harzianum* treatment was lower than the check, forming an intermediate group between the check and the other treatments (Table 1). The post-plant TTC-L treatment when used alone had no effect on recovery of the fungus. This was probably due to the low concentration (500 mg/ml) of TTC-L used. The pre- and post-plant TTC-L treatment, however, was consistently effective at both soil depths evaluated. Additional studies to determine recovery of *A. mellea* on roots segments is being repeated for the 1994-95 season.

Percent survival and decline rating of replant trees after soil fumigation treatments. After 10 months, tree survival slightly decreased in pre-plant or pre- and post-plant TTC-L treated soil when compared to trees grown in soil fumigated with methyl bromide (pre-plant only) or water treatments (Table 2). No difference was observed in decline rating of these treatments. In trees treated only with post-plant TTC-L, a significant reduction (50%) in tree survival and in tree decline was observed. *Armillaria* was not recovered during isolations from tree roots that died. Thus, the percent survival data suggests that the TTC treatments maybe slightly phytotoxic to young replanted trees. Interestingly, the post-plant treatment of TTC was the most injurious (Table 2). Perhaps soil conditions such as temperature and moisture levels may predispose almond trees to injury when post-plant applications of fumigants are used.

Table 2. Percent survival and disease severity of almond replants (Lovell peach rootstock) one year after fumigation treatments with tetrathiocarbonate (TTC) or methyl bromide.[1]

Treatment	No. of Trees	Percent Survival[2]	Severity Rating
Preplant TTC	18	83.33 a	0.84 a
Pre- + Postplant TTC	18	88.88 a	0.50 a
Postplant TTC	18	50.00 b	2.61 b
Preplant TTC + *Trichoderma*[4]	18	94.44 a	0.22 a
Preplant Methyl Bromide	18	100.00 a	0.16 a
Check	17	100.00 a	0.11 a

[1] - Fumigation treatments were: Preplant TTC - 3850 *ug*/ml; Postplant TTC - 500 *ug*/ml; Pre- + Postplant TTC; Methyl bromide (454 g/site); and check (nontreated).

[2] - Percent survival was the percentage of the number of trees alive of the total number of trees treated. Values followed by the same letter are not significantly different ($P > 0.05$) based on general linear model and least significant difference mean separation (LSD).

[3] - Severity rating: 0=healthy; 1=chlorotic leaves; 2=chlorotic leaves and thin canopy; 3= Thin canopy and dieback; and 4=Tree death.

[4] - Cultures of *Trichoderma harzianum* were grown in vitro on molasses, yeast extract media, applied in a cellulose gel as a root dip to replant trees, and as a soil injection method 20-25 cm within 30 cm of replant crowns.

Percent survival and decline rating of mature infected trees treated with TTC. Percent survival and decline rating of mature trees treated with TTC or water were not significantly different six months after treatment; although, a trend was observed with a higher percent survival and a lower decline rating for trees treated with TTC. Of the 10 trees that were treated for each treatment, 80% survived in the TTC treatment whereas, 50% survived in the check. Severity rating for the two treatments were similar at 1.75 and 2.6 for the TTC and check treatments, respectively. Although the therapeutic treatments did not appear to be phytotoxic to the mature, infected trees still declined whether treated or not with TTC. Perhaps the additional applications of TTC-L in would help to maintain tree survival. Eradication of the pathogen or prevention of death from trees extensively colonized by the fungus is not promising with the use of TTC.

Recovery of *Trichoderma* Species from Soil. Six and 12 months after soil fumigation treatments, no statistical difference was observed in the mean number of colonies of *Trichoderma* or other fungal species between check and soils fumigated with methyl bromide or TTC-L (Table 3). Colonies that grew on the selective medium from the two dilution's were proportional. Numerically, however, a higher average number of *Trichoderma* colonies was found in the treatment where *T. harzianum* was applied to roots and injected into soil surrounding (30 cm radius form tree crown) the replant trees. Variation in the replications prevented separation of treatments, although the highest number of *Trichoderma* colonies were in the pre-plant + *T. harzianum*.

Table 3. Number of colonies of *Trichoderma* species and other fungi from soil 6 and 12 months after fumigation and 10 months after almond trees (Lovell peach rootstock) were treated with or without *Trichoderma harzianum*.[1]

Treatment	Mean No. of Colonies after 6 months *Trichoderma* spp.	Other Colonies[2]	Mean No. of Colonies after 12 months *Trichoderma* spp.	Other Colonies[2]
Pre- + Postplant TTC	6.01 a	43.34 a	2.80 a	17.80 a
Preplant TTC + *Trichoderma*[3]	9.00 a	35.16 a	5.40 a	21.70 a
Preplant Methyl Bromide	5.34 a	21.99 a	2.90 a	26.50 a
Check	5.27 a	32.33 a	3.20 a	24.10 a

[1] - Fumigation treatments were: Preplant TTC (3850 *ug*/ml) + Postplant TTC (500 *ug*/ml); Preplant TTC + *T. harzianum*; Methyl bromide (454 g/site); and check (nontreated).

[2] - Values are the means of colonies of Trichoderma spp. or other fungi on 6 plates of TSM per replication. For each treatment, 10 replications were used. Values followed by the same letter are not significantly different ($P > 0.05$) based on general linear model and least significant difference mean separation (LSD).

[3] - Cultures of *Trichoderma harzianum* were grown in vitro on molasses, yeast extract media, applied in a cellulose gel as a root dip to replant trees, and as a soil injection method 20-25 cm within 30 cm of replant crowns.

Effects of direct application of fumigants to almond stumps. The effect of fumigants to eradicate the fungus from infected trees by direct application of the chemicals to tree stumps was evaluated. After 1 year, the fungus was recovered from roots of all of the fumigation treatments and from the crown of most samples evaluated. Thus, no differences were observed between fumigation treatments and the nontreated check. Although this method of fumigant application has been reported for treatment of conifers with Armillaria root rot in conifer forest ecosystems, this method did not reduce the recovery of *A. mellea* from infected almond trees.

In Vitro Evaluations of Alamo. Growth occurred at 0.01 and 0.001 (*u*g/ml), however, inhibition of growth occurred at 0.1 *u*g/ml and no growth occurred at 1 *u*g/ml (Table 4). Growth at 0.001 *u*g/ml was similar to that of the check. Results were similar for the other isolates and experiments. The model of the regression for Log_{10} transformed data was significant ($P < 0.05$) with an R^2 value of 0.88 ($Y = 1.42 - 1.1X$).

Table 4. Growth of *Armillaria* on media containing propiconazole.

Conc. (ppm)	Mean	Std Dev.
0.000	35.7	1.7
0.001	33.0	2.0
0.01	29.3	4.9
0.1	10.0	4.1
1.0	2.25*	1.3

* = Fuzzing from original plug, no growth on medium.

Based on laboratory evaluations, propiconazole was inhibitory at 0.1 *u*g/ml and prevented growth at 1.0 *u*g/ml. At these concentrations, propiconazole appears fungistatic since mycelial growth occurred on the plug of inoculum at 1.0 *u*g/ml. The log_{10} of fungicide concentration was regressed on the probit of the percent reduction of growth. The model was significant ($P < 0.05$) and linear ($R^2 = 0.88$). The equation was $Y = -0.82 + 0.5X$ and the EC_{50} was calculated as 0.15 *u*g/ml. Propiconazole was completely inhibitory at concentrations of 10 *u*g/ml.

In Situ Evaluations of Alamo. Preliminary field work was encouraging, however, larger scale tests are required to fully evaluate this product for therapeutic treatment of trees infected with *Armillaria* root rot. On infected almond, control is dependent on preventing the fungus from reaching the crown and lower trunk. In June 1992 and 1993, therapeutic passive injections of Alamo (400 *u*g a.i./ml) were made in five, 7- or 8-yr old almond trees (Lovell peach rootstock) infected with the fungus. On a rating of 0 to 4 (4=death), initial mean disease severity (DS) ratings were 1.8 for treated and 2.0 for nontreated trees. In 1992, after 5 months, all treated trees were alive (DS=1.8), whereas 4 of 5 nontreated trees were dead (DS=3.6) (Table 5). In 1993, the same trees were rated (DS=2.0), treated again in June, paired with non-treated trees (DS=2.0), and evaluated after 5 months. Four of the five treated trees (DS=2.5) survived another season; whereas four of the five trees in the second set of non-treated trees died (DS=3.8) (Table 5). Foliar applications and preharvest soil drenches of propiconazole (Orbit 3.6EC, Alamo 1.1EC) are currently being evaluated as a non-injurious application method for preventative control and as a possible compliment for fumigants for control of this disease.

Table 5. Disease severity rating and mortality of treated and check trees 5 months after treatment.

	1992		1993	
Treatment	Disease Severity[1]	Mortality[2]	Disease Severity	Mortality
Alamo	1.8	0%	2.5	20%
Check	3.6	60%	3.8	80%

[1] - Severity ratings were based on the scale: 0 = healthy; 1 = 25% dieback; 2 = 50% dieback and yellowing of leaves; 3 = 75% dieback and some scaffold limbs dead; and 4 = tree death.

[2] - Mortality equals the number of trees that died of the total trees evaluated (n = 5/treatment).

Protection of pruning wounds with systemic fungicides. In evaluations of systemic fungicides for control of wood decay organisms in pruning wounds, propiconazole, imazilil, Nectec®, and triadimefon were either applied before or after inoculation of stub cut pruning wounds with the white rot fungus, *Trametes versicolor*. These experiments were initiated in the fall season when pruning of almond is commonly done. In wounds treated and then inoculated, no decay developed in any of the treatments including the non-treated check. In all of these treatments, natural drying and wound healing prevented the establishment of decay. In wounds inoculated first and then treated, decay was most severe in treatments that used sealant materials (e.g. Nectec®). These treatments prevented drying of the wounds and had the smallest zones of discoloration (a host reaction to injury). Currently, both experiments are being repeated. Thus, the use of fungicides or fungicidal sealants to treat tree wounds is questionable under dry conditions where rapid wound healing occurs during fall pruning of almond trees in California.

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