Use of Boron for the Control of Eutypa Dieback of Grapevines

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ABSTRACT


Eutypa dieback is a perennial canker disease of grapevine (Vitis vinifera) caused by Eutypa lata. The fungus produces ascospores, which infect grapevines through pruning wounds during the dormant season. Management of the disease has been achieved with fungicidal applications during the dormant period. However, no effective fungicide was available for this purpose after Benlate was withdrawn from the market. Boric acid (17.5% a.i. boron), a potential alternative to Benlate, was tested in the present study against E. lata. The EC50 values for inhibition of mycelial growth and ascospore germination were 125 and 475 µg of boric acid per ml (22 and 83 µg a.i./ml), respectively. Two boron-based treatments were developed and tested in vitro and in four field trials during 2001 to 2003. One product, biopaste, contained 5% boric acid (8.75 mg a.i./ml) in a commercial paste. The second product, biofill, contained 5% boric acid in a spore suspension of Cladosporium herbarum. Both products significantly reduced disease in vitro and in field trials in comparison with a water control treatment. Boron was not found to accumulate in leaves and shoots, but bud failure at the first node below the treated wound occurred at a higher rate than in untreated vines.

Additional keywords: fungicide screening, wood decay

Eutypa dieback is a severe disease of grapevine (Vitis vinifera L.) caused by the fungus Eutypa lata (Pers.:Fr.) Tul. & C. Tul. (syn. E. armeniacae Hansf. & M.V. Carter). The pathogen impacts grapevines by reducing vegetative growth and fruit yield (22). Siebert (34) reported that the overall loss in net income for wine grapes in California was estimated to be over $260 million per annum.

E. lata infects grapevines through pruning wounds during the dormant season by means of ascospores released from perithecia after rainfall. The disease slowly kills the vine over several years (3). Pycnidia (anamorph Libertella blepharis A.L. Smith) are also produced by E. lata, but conidia or possible spermatia are not infectious (4). Several woody plant species are potential hosts for E. lata (5). The presence of E. lata perithecial stroma within and around Californian vineyards on many of these hosts has been reported, and these serve as a natural reservoir for E. lata inoculum (F. Trouillas, personal communication).

Disease management can be partially achieved by late pruning of vineyards. Pruning wounds can be susceptible for as long as 7 weeks (8), but the length of this period varies with the time of pruning and the age of the pruning cut (8, 25, 27). Munkvold and Marois (25) determined that wound susceptibility to E. lata declined faster with high degree-day accumulation, due to wound healing by deposition of polymerized phenolic compounds in the opened wood vessels and concomitant establishment of an epiphytic microbial population on the wound surface. However, the large acreage of vineyards in California makes late pruning impractical for most growers. Also, late rains in the dormant season can trigger ascospore release and increase the risks of infection of newly pruned vineyards. Disease management is usually achieved by treating pruning wounds with fungicides or biological control agents (6, 12, 14, 21, 23, 24). Benlate (DuPont de Nemours & Co., Wilmington, DE) was registered for E. lata control for 30 years, and field trials showed Benlate efficacy in preventing Eutypa dieback (14, 21, 23). However, long-term protection of pruning wounds was not achieved because the product did not persist in woody tissue (7, 28). Therefore, several applications of Benlate were required to provide protection until complete healing of the wound, which was costly to growers.

Biocontrol agents were tested as an alternative method for control of E. lata. Bacillus subtilis (12), Fusarium lateritium Nees: Fr., and Cladosporium herbarum (Pers.: Fr.) Link (24) showed some potential activity in limiting the establishment of the pathogen. Unlike chemical applications, which have an immediate effect, maximum protection from biocontrol agents requires colonization of the surface of the wound. Thus, there is a window of susceptibility after treatment, until the biocontrol agent is established well enough to prevent development of E. lata in the wounded tissue (6).

Boron (B) is an essential element for higher plants (1) and can be applied as soil dressings or as foliar sprays to limit boron deficiency (19, 11). Boron was also found to be active against several wood decay fungi (10, 16, 18, 33, 35), and it is used in the timber industry to protect wood from termites and fungi (18) and in forestry to prevent infection of conifers by Heterobasidion annosum (Fr.) Bref. (=Fomes annosus (Fr.) Karst.) (16, 33, 35). Irelan et al. (17) also showed the efficacy of boric acid treatment to control infection of pruning wounds by E. lata in a field trial.

Our objective was to develop a control method for E. lata that is easily used by growers. The effect of boron on E. lata ascospore germination and mycelial growth was evaluated, and boron-based products were developed and tested in vitro and in field trials. Boron phytotoxicity was also evaluated, and boron levels were measured in grape leaves and shoots.

MATERIALS AND METHODS

In vitro evaluation of boron effect on E. lata growth and germination. An E. lata isolate (E31), obtained from a canker of grapevine cv. Chenin Blanc, was grown on potato dextrose agar (PDA). A 1-cm-diameter plug of mycelial agar was obtained from the actively growing margin of the colony and placed in the center of a petri plate supplemented with boric acid. Boric acid (17.5% a.i. boron) was added to PDA after autoclaving to achieve final concentrations of 0, 100, 200, 300, 400, and 500 µg/ml. Five replicates were used per boric acid concentration. Plates were incubated at room temperature (20 ± 2°C) in the dark for 7 days. The radial growth was measured as the distance from the edge of the original plug to the outer margin of the colony. Two measurements were taken per colony.

Perithecial stroma of E. lata were collected from a vineyard in Napa County, CA, and identified based on the morphological descriptions of Rappaz (29). Ascospores were obtained from perithecial stroma as described by Carter (4). Concentration was adjusted to approximately 500 ascospores per microliter with a hemacy-

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percent inhibition for each fungicide correlated by a regression of a probit of the mycelial growth of E. lata were calculated by a regression of a probit of the percent inhibition for each fungicide concentration over the log_{10} of the fungicide concentration (13).

Formulation and in vitro evaluation of boron-based treatments. Boric acid efficacy was tested in vitro, in order to determine the concentration of boronic acid at which control of E. lata could be achieved (30). One-year-old dormant grape canes cv. Cabernet Sauvignon were pruned, cut to blocks of 4 cm in length, and sterilized by autoclaving twice. Wood blocks were further dipped in sterile water for 15 min and then dipped in boronic acid solutions for another 15 min under a laminar flow hood. Boronic acid solutions were prepared by dissolving boracic acid in sterile hot water at final concentrations of 0, 0.1, 0.5, 1, 5, and 10% (wt/vol). Control wood blocks were dipped in water only. Wood blocks were then blotted on a sterile paper towel to remove excess liquid. French squares were cut from 250 ml (Fisher Scientific, Pittsburg, PA) bottles and filled with 15 ml of PDA were autoclaved and laid flat on their side for cooling. A plug of agar from the edge of an E. lata culture was transferred into bottles and allowed to grow for 5 days prior to inserting the wood blocks into the bottles. Bottles were incubated for 4 weeks at room temperature (20 ± 2°C), after which blocks were removed from the bottle and bark was stripped off. The wood blocks were surface sterilized by flaming, split open longitudinally, and five chips of wood (3 × 2 × 2 mm) were removed with the sterile blade of a knife and plated on PDA, with two replicates per wood block. The wood chips inoculated onto PDA represented roughly 1% of the total wood block. Petri plates were incubated at room temperature (20 ± 2°C) for a week. Boron treatments were evaluated as the percentage of wood blocks from which E. lata was recovered.

Based on the above results, a liquid and a paste boron formulation were developed. Biopesticide consisted of a mixture of boric acid (5% wt/vol) and a spore suspension of C. herbarum that had been challenged until the fungus could grow and survive at this boron concentration (10,000 spores per µl). Biopaste consisted of a mixture of boracic acid (5% wt/vol) and a commercial paste (latex paint) used on trees as wound dressing and grafting seal (Doc Farwell’s, Wenatchee, WA). Six treatments consisting of the water control, 5% boronic acid, biopaste, biopesticide, C. herbarum, and boron-free paste were evaluated simultaneously in vitro for control of E. lata as described previously (30).

Field evaluation of boron-based treatments. Trials were conducted during the 2001–2002 growing season in Yolo and San Joaquin counties and during 2002–2003 in Napa and San Joaquin counties in California. Grapevines were pruned in November to two buds, and treatments were sprayed (liquid) or painted (paste) onto pruning wounds. The treatments included water control, 5% boron, biopaste, biopesticide, C. herbarum, and boron-free paste. All six treatments were evaluated by inoculating approximately 1,000 ascospores of E. lata directly onto pruning wounds at 1 and 10 to 12 days after treatment. Uninoculated pruning wounds were also included in order to determine the rate of natural disease occurrence in each vineyard. Twenty spurs were used per treatment and per time of inoculation in Yolo and San Joaquin counties during the two growing seasons, and 50 spurs were used in the Napa County field trial. Spurs were harvested the following dormant season to determine the percent recovery of E. lata from the wood. The isolation techniques were identical to that described above for the in vitro assay. Results were represented as the mean percentage of infected spurs and the mean percentage of infected spurs at two times of inoculation. Percent disease control, Pdc, was calculated by Pdc = 100(C1 – C2)/C1, in which C1 is the proportion of infected inoculated control spurs and C2 is the proportion of infected treated spurs. Mean percent disease control was calculated for each treatment at both times of inoculation and transformed to the arcsine square root of the fractional value. Each field trial was treated as a block (replication), and the data were analyzed using a two-way ANOVA. Tukey’s test was used to determine which means were significantly different. All data analysis was performed using SAS (version 8.2, SAS Institute, Cary, NC).

RESULTS

The EC50 values for inhibition of mycelial growth and ascospore germination were 125 and 475 µg of boracic acid per ml, which corresponded to 22 and 83 µg of boron per ml, respectively. E. lata grew directly on the water-treated wood blocks by producing a white flocculent mycelium. The isolation techniques performed yielded 100% recovery of E. lata from the control wood blocks. Fungal growth on wood blocks was not prevented with boron acid concentrations below or equal to 10 mg/ml (1.75 mg a.i/ml) (data not shown), while at 50 mg/ml (8.75 mg a.i/ml), growth generally did not occur on the grape wood blocks and E. lata was recovered from only 10% of wood blocks. Therefore, the concentration of 50 mg/ml was chosen to develop the two boron-based treatments, biopaste and biopesticide. Our results indicated full protection of wood blocks for 4 weeks in vitro when treated with biopaste and biopesticide. However, E. lata totally colonized wood blocks when they were only treated with C. herbarum or boron-free paste (Fig. 1).

Treatment efficacies were evaluated based on data obtained from four field trials, and results are summarized in Table 1. Natural infection of control grapevines by E. lata ascospores was very low. However, ascospore inoculation of E. lata was successful and averaged 64% in the first inoculation, but decreased to 32% in the second inoculation 10 to 12 days following pruning. No significant difference was observed between inoculated control, boron-free paste, and C. herbarum at either inoculation time. Boron-based treatments significantly reduced infection by E. lata at both inoculation times.

Although boron levels varied depending upon year, vineyard location, and plant

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This text is a representative sample of content that could be found in a scientific journal article, discussing the evaluation of boron-based treatments for grapevine disease control.
Boron has previously been shown to affect many wood rotting fungi (10,16,18,33,35), and is currently used in timber (18) and in forest (16,33,35) industries. Boron products could potentially be used in viticulture to protect pruning wounds from E. lata. Boron-based products resulted in over 75% disease control 10 to 12 days following treatments. However, pruning wound susceptibility was reported to last several weeks (8,25), and additional data are needed to evaluate the protective efficacy of boron products over that time period. The percent decrease of infection of inoculated control spurs from 64 to 32% after 10 to 12 days indicated that pruning wound susceptibility was affected by the age of the wound. Munkvold and Marois (25) also demonstrated that wound susceptibility was affected by the age of the wound as well as the time of pruning. However, they also concluded that wound susceptibility was not affected by the age of the wood. Therefore, boron-based products can be applied on small wounds of 1-year-old canes and on larger wounds of older wood and will provide the same effective protection against E. lata.

The mechanisms by which boron limits fungal growth have been studied in only a few pathosystems. Bowen and Gauch (2) reported that high levels of boron inhibited the growth of Saccharomyces cerevisiae and Penicillium chrysogenum by inhibiting the glycolysis pathway. Aldolase was suspected to be the target of toxic levels of boron, making the fungi unable to utilize carbohydrates at a sufficient rate to maintain metabolic processes involved in growth and reproduction. Parker et al. (26) identified a mechanism by which Paecilomyces variotii could overcome the toxicity of boron by overproducing β-D-1,4-glucosidase, thereby making more glucose available from the wood substrate for fungal metabolism. E. lata was determined to primarily deplete noncellulosic glucose of the hemicellulose fraction of the grapevine cell walls (31). However, further studies are needed to determine if dysfunction of glycolysis occurred with E. lata when treated with boron and/or if other mechanisms are involved in limiting fungal development.

Biocontrol agents tested as alternatives to fungicides have had mixed results (6,24). Munkvold and Marois (24) observed a reduced infection rate in field trials when E. lata inoculation was conducted 14 days after treatment with C. herbarum, but our results did not show a reduction of infection rate when E. lata was inoculated 10 to 12 days after treatment with C. herbarum. The biology of C. herbarum has not been studied extensively, and the optimal conditions required for this organism to colonize pruning wounds may not always be reached under natural conditions, thereby offering limited control of E. lata.

The combination of fungicide and biocontrol agents to improve the duration and/or the efficacy of control of E. lata has also achieved limited success. Carter and Price (6) combined Benlate with Fusarium lateritium, but detected no significant differences in comparison with benomyl or F. lateritium treatments alone. McMahan et al. (19) also isolated a benomyl-resistant strain of F. lateritium, but field results are lacking to fully evaluate this combination. C. herbarum was able to grow on PDA medium amended with 5% boric acid (P. E. Rolshausen, unpublished). However, the combination of C. herbarum with boric acid did not significantly improved disease control over time, in comparison to boric acid alone.

Boron is an essential element for plants. It is required in developing leaves, stems, and flowers (1). Plants take up boron through their root systems and leaves, and foliar applications and soil dressings of boron are often used by growers to limit boron deficiencies and improve fruit set.

**DISCUSSION**

Our results clearly demonstrated the fungicidal activity of boron against E. lata.
Christensen (9) reported that both boron deficiencies and toxicities were found in Californian vineyards due to soil characteristics, which would partially explain the field-to-field variability in boron levels observed in our trials. Our results also indicated higher boron levels in leaves than in shoots of grapevine. Gimmler et al. (15) also found accumulation of boron mostly in leaves sometimes to the level of phytotoxicity. The toxic threshold for boron in grapevines was reported at 100 µg/g of dry matter (15). This level was exceeded in the leaves of grapevines in one of our trials in Yolo County. However, no phytotoxicity was observed on the foliage of grapevines in this or any other trial in these studies. Boron levels were generally much lower in shoots than in leaves, and therefore the overall level of boron in dry matter may be below the phytotoxic level as reported by Gimmler et al. (15). Our observations indicated that the bud located at the first node below the pruning wound sometimes failed to push. Dye et al. (11) also observed bud-drop on nectarine and peach trees treated with toxic amounts of boron-based fertilizers. Analysis of boron levels of grapevines treated with biopaste and bioshield did not indicate an accumulation in leaves and shoots. However, limited movement of boron from the pruning wound to the first bud may have occurred and resulted in the failure of the bud to push. Further study is needed to confirm the effect of boron on bud failure of grapevine and understand its mechanism of action. Boron-based fungicide formulations could be improved to increase the duration of control and to limit its putative phytotoxic effect. Kartal et al. (18) showed improved performances of Tim-bor (US Borax Inc., Valencia, CA) against termites, Coptotermes formosanus; a white-rot fungus, Trametes versicolor; and the brown-rot fungus, Fomitopsis palustris, by limiting leachability of boron. External application of boron does not adhere to the cell walls of wood, and several system fixations have been devised to modify the wood properties and limit the natural diffusibility and leachability of boron on wood surfaces, and therefore increase protection against wood decay organisms.

Treatment of pruning wounds with boron offers an effective, economical, and environmentally safe management strategy to control *E. lata* in vineyards. However, formulations have to be optimized in order to increase the duration of control on the surface of wounds and limit putative effect on bud failure of grapevines. Potentially, boron-based treatments could be used on several cultivated crops on which *E. lata* was found to be pathogenic (5).

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