Inoculation Technique, Infection Development and Efficacy of Fungicides Against *Glomerella cingulata* the Causal Agent of Camellia Dieback

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Introduction: Camellia dieback, caused by *Glomerella cingulata* is one of the most damaging diseases of camellias grown in nurseries, landscape plantings and private gardens (Baxter and Fagan, 1974; Baxter et al, 1979; Baxter et al, 1982; Dickens and Cook, 1990; Jeffers and Baxter, 2001). The disease is increasingly important in container grown camellias, especially *C. sasanqua* and *C. japonica* rendering enormous economic losses (Jeffers and Baxter, 2001; Hagan and Muellen, 2002). *G. cingulata* produces a noticeably dulling or yellowing of the normally shiny deep green leaves on the diseased shoots. The dull green foliage wilts, turns yellow, and finally turns reddish brown. Well-defined, sunken cankers with a prominent callus ring develop at the base of the dead shoots. The cankers will continue to enlarge until the scaffold limbs or root collar is girdled. Shoot dieback can appear at any time during the growing season. If left unmanaged the pathogen often kills the plant. The efficacy and effectiveness of fungicides against camellia dieback has not been clearly established (Hagan and Muellen, 2002), additionally newer chemistries are available and had not been tested. In addition to well know fungicides (Thiophanate methyl, propiconazole, etc) or other protectants, strobilurins (Azoxystrobin and Trifloxystrobin) are now available. Of particular interest to be tested is Actigard, a plant activator, a systemic compound used for the control of fungal and bacterial diseases in vegetables and tobacco. Actigard does not act on the pathogen, rather induces the natural host plant resistance. Knowing the efficacy of these fungicides and plant activators will lead to a better management of the dieback pathogen and prevent diseases losses. Objectives: 1) To test the efficacy of several know fungicides to control *G. cigulata.* 2) To test the efficacy of several newer fungicides to control including Azoxystrobin (Heritage) and Trifloxystrobin (Compass) and Actigard against *G. cingulata.*

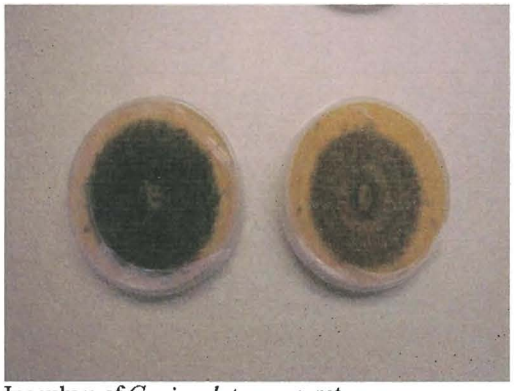

Camellia Dieback caused *G. cingulata*


Camellia Dieback caused by *G. cingulata* –artificial inoculation

Development of an efficient inoculation technique and infection development of Glomerella cingulata on Camellia sasanqua and Camellia japonica.

In order to have one standardized inoculum preparation method and an effective inoculation technique that would insure infection of *G. cingulata* on camellias, several experiments were performed in Griffin prior to perform the final CANR experiments.

Inoculum preparation: The fungus was isolated from infected tissue using standard laboratory techniques. Inoculum of *G. cingulata* was prepared and evaluated in two agar media for the maximum production of spores. Carrot juice agar was prepared as described in Baxter et al, 1982, after 5 days, the fungal colony grown on Petri plates was either dry-scraped using a sterilized scalpel (or a sterilized glass slide) or wet-scraped using distilled sterile water. Four days after the scrape, the inoculum was observed at the microscope and the spores counted. An alternative medium was also evaluated; PD agar amended with chloramphenicol was used to grow the pathogen. The fungal colony was either dry or wet-scrapped and evaluated as previously described. Both media produced abundant mycelium and spores. Additional experiments indicated that "hybrid" medium, carrot juice agar which was amended with an antibiotic (chloramphenicol) gave the fastest growth and the mayor number of spores when compared with other media.



Inoculum of *G. cingulata* on carrot agar

G. cingulata inoculum preparation

Inoculation technique: Due to the suggestions of the CANR board on the inoculation technique to be used and some discrepancies with the inoculation method described widely in the literature, we decided to evaluate the effectiveness of several inoculation methods. Three inoculation techniques were tested: a) mechanical injury with an scalpel followed by an agar plug deposition on injury (Baxter et al, 1982); b) leaf removal followed by a sporidial spray and c) injection using a hypodermic syringe. These three techniques were performed evaluated. Plants were placed on a high humidity regime for *G. cingulata* infection. Inoculated and fungicide treated plants were then arranged in a randomized complete block design, using four single replications per treatment. Lesion size and extent of damage on branches and foliage were recorded. The three methods provide satisfactory infection of *G. cingulata* on camellias, however visual inspection and data showed that the mechanical injury with an scalpel followed by an agar plug deposition on injury and the leaf removal followed by a sporidial spray gave larger cankers and more numbers of branch dieback, therefore these two methods were selected. No statistics were performed in these experiments. However data clearly shows differences.

Screening of inoculation methods

Screening of inoculation methods

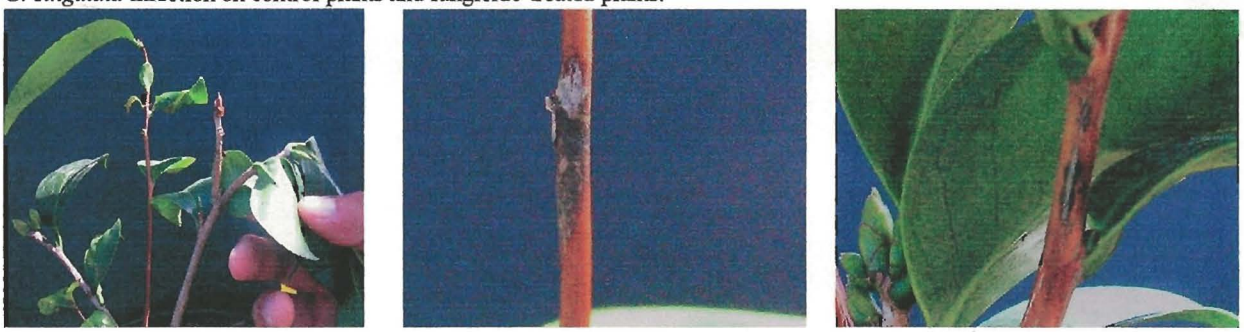
Efficacy of fungicides against *Glomerella cingulata* the causal agent of camellia dieback, 2004

The evaluation was conducted in a polyhouse at the Center for Applied Nursery Research located in Dearing, GA. Camellia plants were grown in a 8 inch plastic containers containing 6:1 pine bark:sand potting mix amended with lime 4 lb/cu yd; micromax 1.5 lb/cu yd; gypsum 1.5 lb/cu yd and talstar 2 lb cu/yd. Plants were irrigated as needed using overhead irrigation throughout the study. Plants were fertilized with osmocote 18-8-8. which was incorporated into the potting mix. Supplemental fertilization was applied as needed. The fungicide evaluation was initiated on 27 Aug 04. Eight leaves from each plant were removed and fungicide treatments were applied. For this purpose Azoxystrobin (Heritage) at 0.4 oz/100 gal; Thiophanate methyl (Cleary's 3336) at 24 oz/100 gal; Propiconazole (Banner Maxx) 8 oz/100 gal; Trifloxystrobin (Compass) 0.4 oz/100 gal and Actigard 1 oz/100 gal were applied to run off using a hand sprayer, the product was left to air dry and the procedure was repeated once. Five incisions were done per plants using a scalpel and a 1 cm square agar media containing *G. cingulata* was carefully placed in the incision. Inoculation sites were identified using twist on plastic wires. Additionally, a mycelium and spore suspension was sprayed thoroughly on each plant. To obtain this inoculum, *G. cingulata* was inoculated on carrot agar plates, grown for 4 days at a 12 hrs dark-light regime, after this time plates were dry scraped using a sterile microscope glass slide to stimulate spore production. Plates were incubated for an extra five days. At the time of inoculation on plants, plates were scraped using sterile distilled water and a microscope slide. Mycelium and spore mass was passed through a cheese cloth mesh and inoculum was collected into a hand held sprayer. Inoculated and fungicide treated plants were then arranged in a randomized complete block design, using four single replications per treatment. Control treatments included pathogen-free camellia plant and a non-treated but pathogen amended check. Plants were then placed on a high humidity regime (10 days using a 10 second mist every 10 min) for *G. cingulata* infection

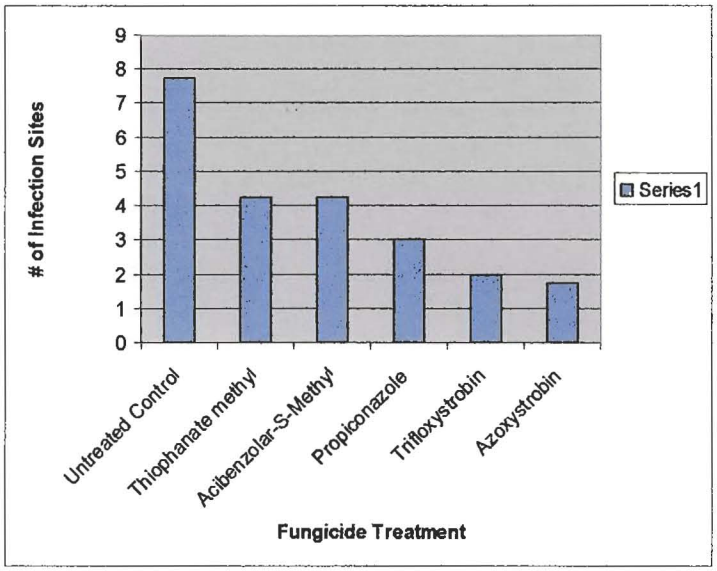
Twenty three days post-inoculation the experiment was evaluated and the disease rating was based on 1) Leaves with marginal lesions, 2) number of visible cankers or darkened stems and 3) lesions with visible black fruiting bodies (perithecia). Data was analyzed using the PROC GLM procedure of SAS and means were separated using Fisher's protected least significant difference (LSD) at P=0.05.

Significantly less infection sites were observed with Azoxystrobin (Heritage) 0.4 oz/100 gal and Trifloxystrobin (Compass) 0.4 oz/100 gal. Propiconazole (Banner Maxx) 8 oz/100 gal. gave a mean of infecting sites of 3.00 and was significantly better than the results given by Thiophanate methyl (Cleary's 3336) 24 oz/100 gal and Actigard 1 oz/100 gal. All treatment had significantly less infection sites than the untreated control. For recommendation purposes the best 3 fungicides were Azoxystrobin, Trifloxystrobin and Propiconazole in that order.

G. cingulata infection on control plants and fungicide-treated plants.



Treatment and rate	Application Method	# of infection sites
Untreated check.....	-------	7.75 a
Azoxystrobin (Heritage) 0.4 oz/100 gal	Foliar spray	1.75 c
Thiophanate methyl (Cleary's 3336) 24 oz/100 gal	Foliar spray	4.25 b
Propiconazole (Banner Maxx) 8 oz/100 gal.	Foliar spray	3.00 bc
Trifloxystrobin (Compass) 0.4 oz/100 gal	Foliar spray	2.00 c
Actigard 1 oz/100 gal	Foliar spray	4.25 b



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