Dip Treatment for Control of Blackstem on *Populus* Cuttings

J. A. WALLA, Research Analyst, and R. W. STACK, Associate Professor, Department of Plant Pathology, North Dakota State University, Fargo 58105

ABSTRACT

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Blackstem of *Populus* cuttings has reduced production of salable plants by more than 50% in a North Dakota forest nursery. Isolations from cuttings indicate that *Cytospora chrysosperma* is the primary pathogen. Inoculum or asymptomatic infections are present on cuttings before winter storage. Most cankers develop from cut ends of cuttings. A dip treatment in thiram before winter storage increased production of salable plants by more than 50% compared with the control.

Additional key words: Northwest poplar, Norway poplar

Cottonwood and hybrid poplars (*Populus* spp.) are planted extensively in the northern Great Plains to shelter fields and farms. Planting stock is obtained primarily from state and federal forest nurseries.

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Production of poplar stock for shelterbelt plantings takes about 18 mo from the time cuttings are taken from stock plants until plants are distributed (Fig. 1). Stems from stock plants are cut 5–8 cm above the ground line in late fall, after they have hardened off for the winter. The stems are cut into 16–20 cm sections (cuttings) and those with a diameter between 5 and 19 mm are stored over winter in boxes or bags in covered insulated pits. Winter storage pits are prepared to keep the cuttings frozen until late spring and to prevent rapid temperature fluctuations. Water is run onto the pit floor and allowed to freeze. Cuttings in boxes or bags are placed on the ice, snow is packed around the containers, and straw is piled on top of the snow to fill the pit. The cuttings are planted the following spring (upright, with tops 2–3 cm below ground line, 10 cuttings in 30 cm) to allow root and shoot development. The plants are left in place over winter and then dug the following spring for planting in shelterbelts (17).

Disease loss in cuttings is a major limiting factor in nursery production of *Populus* planting stock. Blackstem is often cited as the cause of poplar mortality in nurseries (2,5,7–9,11). Gray

et al (7) described blackstem symptoms as bark necrosis due to an infectious disease. It is often considered a disease problem on trees of low vigor (1–5, 8–11). Low moisture content is responsible for the low vigor and lack of defense mechanisms (1–4). Several pathogens have been associated with blackstem, including species of *Cytospora*, *Phomopsis*, and *Dothichiza* (1,6,9,15,16). Long (9) and Hubert (8) reported *Cytospora chrysosperma* (Pers.) Fr. as the cause of blackstem of poplars in North Dakota nurseries in 1918 and 1920, respectively.

Our study began in 1977 in response to problems at a forest nursery in North Dakota. The nursery manager estimated 55% losses to disease in 1977 and near 35% in most years. Because of these losses, they were able to supply only one-half of this state's needs. Additional production to supply adjacent states would be possible if the proportion of cuttings lost could be reduced. Our objectives were to determine the causal organism(s), their etiology, and practical control measures. A preliminary report was presented (14).

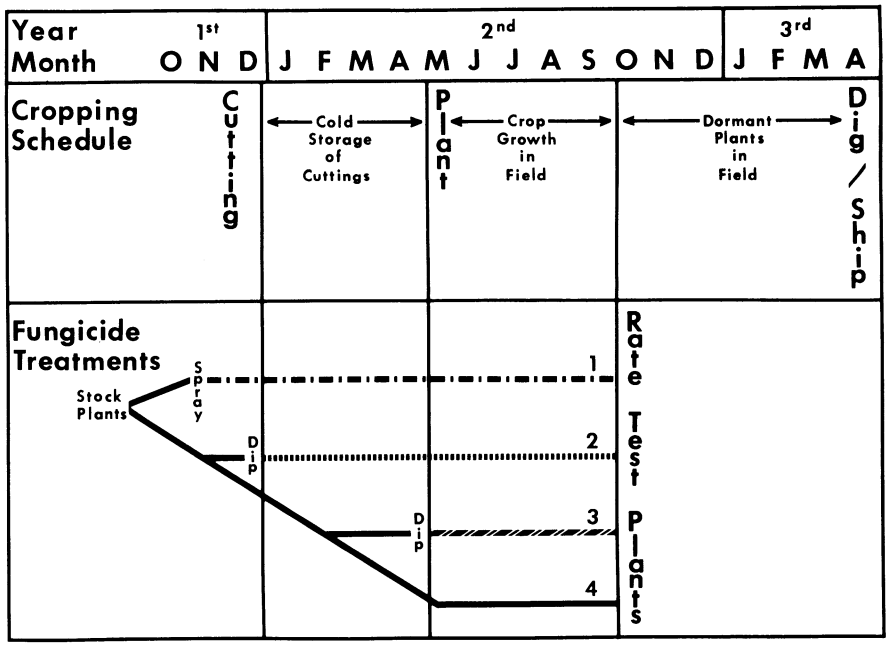

Fig. 1. Cropping schedule and fungicide treatments, used to test blackstem control in poplars. The 18-mo cropping schedule is completed in three calendar years. Percent salable plants were rated in each treatment after the growing season. Thiram (2.25 g[a.i.]/ L of water) was used to: **(1)** spray stock plants to runoff before cutting in fall, **(2)** dip cuttings for 5 min before winter storage, **(3)** dip cuttings for 5 min after winter storage in spring before planting. No thiram applied in treatment **4**.

MATERIALS AND METHODS

Field observations. Cuttings planted in April 1977 were examined in mid-May, 1977. Development of plants was poor, and samples were collected. Stock plants were examined microscopically for pathogenic fungi and sections of tissue were plated on potato-dextrose agar (PDA).

Fungicide tests. To determine when infection occurs and to evaluate canker development and field performance, experiments with stock plants and cuttings treated with fungicide were done during two seasons (Fig. 1). There were four treatments. Treatment 3 was not done in 1977–1978, and treatment 1 was not done in 1978–1979. The fungicide thiram as a 50% wettable powder at 2.25 g (a.i.)/ L of water was used for all treatments. Thiram is registered for use in nurseries and does not inhibit rooting (12,13). Hybrid cultivars Northwest (*Populus deltoides* × *P. balsamifera*) and Norway (*P.* × *canadensis 'Eugeneii'*) are main poplars grown in this nursery and were used in these tests.

Several stock plants of each cultivar were sprayed to runoff with thiram in November before cuttings were taken (treatment 1). Those not sprayed were used for cuttings for treatments 2, 3, and 4. One-thousand cuttings from unsprayed stock plants were dipped in thiram for 5 min (treatment 2). Cuttings from treatments 1 and 2 and untreated cuttings were wet down and stored. Cuttings were stored in plastic-lined wooden boxes in 1977–1978 and in black plastic storage bags in 1978–1979. In May the cuttings were removed from storage and 1,000 untreated cuttings were dipped in thiram for 5 min (treatment 3), after which all cuttings were planted. Cuttings not treated with thiram were the check (treatment 4).

Development of poplars was observed during the growing season. When plants were harvested in September, the number of cuttings killed before and after shoot development were counted, and the diameter of live shoots was recorded. Dead cuttings and cuttings with live shoots less than 5 mm in diameter at ground line were considered unmarketable.

Isolation from cuttings. One-hundred cuttings were taken from each treatment at two times: before winter storage and before spring planting. To find where pathogens were present on cuttings, isolations were made from various parts. Ten cuttings from treatments 1, 2, and 4, collected before 1977–1978 storage, were washed for 5 min in running tap water. Tissue taken from both ends, from buds, and from wounds was plated on PDA.

Canker development. To evaluate canker development in storage, cuttings collected in fall 1977 (before storage) and in spring 1978 (after storage) were rated and isolations were made.

To rate canker development on treated cuttings, asymptomatic cuttings were incubated in closed plastic bags at room temperature (20 C) to allow canker development. After 52 days, percent area cankered and location of cankers were recorded on each cutting and analyzed by the Chi-square test.

Pathogenicity tests. *C. chrysosperma* and *Phomopsis* spp. isolates from infected cuttings were tested by end and inverted-V wound inoculations. Northwest poplar cuttings without apparent cankers or wounds were used. All cuttings were surface-sterilized in 1% NaOCl for 5 min. For end inoculations, 2 cm was cut from the bottom of each cutting with surface-sterilized clippers.

Two-week-old cultures on PDA of four isolates of *C. chrysosperma* and two isolates of a *Phomopsis* sp. were each mixed separately in a blender with 35 ml of distilled water. The freshly cut bottom ends of cuttings were dipped in the blended mycelial inoculum to a depth of

Table 1. Percent salable plants of Northwest and Norway cultivar poplars as affected by fungicide application

	Northwest		Norway	
Treatments	**1977–1978**	**1978–1979**	**1977–1978**	**1978–1979**
Fall spray[a]	48	...	72**	...
Fall dip	55**[b]	52**	78**	42*
Spring dip	...	32	...	37
Control	41	29	52	29

[a] The fungicide was thiram (2.25 g[a.i.]/ L of water).
[b] **, * = significantly different from the control at the 1 or 5% level, respectively. Actual counts were compared by the Chi-square test.

about 2 cm. Cuttings were placed in separate plastic bags and incubated at 18 C for 21 days. The two controls were dipped in sterile PDA or not dipped. There were five cuttings in each of eight treatments, and there were four replications.

For inverted-V wound inoculations, 1.2-cm cuts into wood were made with a surface-sterilized knife blade. A wound was made near each end of each cutting. Inoculations were made by lifting the bark flap and placing a wedge of mycelium from 2-wk-old cultures on PDA under the flap. One isolate each of *C. chrysosperma* and *Phompsis* was used. The control was sterile PDA. Two wounds were made on each of 10 cuttings, in each of the three treatments. Inoculated cuttings were incubated in separate plastic bags at 18 C for 21 days.

RESULTS

Field observations. In beds of poplar cuttings in which losses occurred, many cuttings were all or partly cankered. The most severely affected cuttings did not produce shoots, and less affected cuttings produced stunted or weakened shoots too small for outplanting. Many cuttings produced shoots but few or no roots. Cortical tissue on the cankered cuttings was black and deteriorated to a loose, stringy layer. Isolations from such cankers yielded various common soil fungi, none considered pathogenic to *Populus* spp.

No cankers were observed on live stems (whips) of stock plants. However, fruiting bodies of *C. chrysosperma* and its perfect stage, *Valsa sordida* Nitschke, were found on dead stubs of previous years' stems. *C. chrysosperma* was found on stock plants of all ages.

Isolation from cuttings. Cuttings collected in fall 1977 before winter storage had no apparent cankers. Tissue isolated from asymptomatic ends, buds, and wounds on 10 cuttings in each treatment and cultivar yielded *C. chrysosperma* and a *Phomopsis* sp. Pathogens were isolated from ends on 40% of the cuttings, from buds on 22% of the cuttings, and from wounds on 32% of the cuttings. About one-half of the cuttings had obvious wounds.

C. chrysosperma was isolated from more cuttings than was *Phomopsis* (63 vs. 27%). Both fungi were isolated from some cuttings. There were no detectable fungicide treatment effects on the incidence of either fungus.

Canker development. No cankers were apparent on more than 100 cuttings from each cultivar from each of treatments 1, 2, and 4 from the 1977–1978 experiment collected before winter storage. However, both *C. chrysosperma* and a *Phomopsis* sp. were isolated from asymptomatic ends, buds, and wounds.

After these same cuttings from treatments 1, 2, and 4 were stored at 8 C, 2% had cankers after 39 days, and *C. chrysosperma* was isolated; a total of 6% had cankers after 74 days, and *C. chrysosperma* and a *Phomopsis* sp. were isolated.

Among 939 cuttings from treatments 1, 2, and 4 in the 1977–1978 experiment collected after winter storage, 149 cuttings (16%) had cankers. There were cankers on cuttings of each treatment. Only 8% of Norway cuttings had cankers, however, compared with 24% of Northwest cuttings. *C. chrysosperma* was isolated from these cankers.

Few cankers developed on cuttings stored at 8 C for 3 mo, and there were no differences between treatments. Cankers developed on most of the 22 cuttings from treatments 1, 2, and 4 that were incubated at 20 C. Most cankers developed from ends, but a few developed around buds and wounds. The fall dipped cuttings of both cultivars (treatment 2) had significantly less extensive cankers than the control, and the sprayed cuttings (treatment 1) had significantly more extensive cankers than the control. Most of the Northwest cuttings had more than 25% of the surface area cankered, but most Norway cuttings were less than 25% cankered. *C. chrysosperma* and a *Phomopsis* sp. were the only pathogens isolated from the cankers in this test. *C. chrysosperma* was isolated more frequently than *Phomopsis*.

Planted cuttings. During the two growing seasons, fall dipped cuttings were the healthiest of the poplars developing from planted cuttings. For both cultivars, they were first to break ground, a higher percentage emerged, they had darker green leaves, and they grew faster. In mid-July 1979, Northwest poplars from fall dipped cuttings were 50% taller than plants from the other treatments. Development of plants from the fall spray and spring dip treatments was intermediate. Some cankered cuttings (dead with no shoots or cankered with small live shoots) were found in each treatment.

When plants were dug and rated in September, the fall dipped cuttings had the highest percent salable plants with both cultivars (Table 1), with the fall spray and spring dip intermediate.

Pathogenicity tests. Significantly more cankers developed on three of the four *C. chrysosperma* end-inoculated cuttings than on the fourth *C. chrysosperma* inoculated, the *Phomopsis* inoculated, or the control cuttings. *Phomopsis* isolates did not cause cankers but could be reisolated from wood on the inoculated cuttings. Cultural characteristics of fungi reisolated from the cuttings in each treatment in one replication were easily matched with the original isolates used for inoculum in all except the one *C. chrysosperma* isolate that was not pathogenic.

Cankers on inverted-V wounded cuttings developed from 100% of wounds on cuttings inoculated with *C. chrysosperma* but not from wounds on the *Phomopsis* inoculated or control cuttings.

DISCUSSION

C. chrysosperma and a *Phomopsis* sp. were the pathogens isolated from cuttings. We consider the primary pathogen to be *C. chrysosperma* because of its much greater incidence on cankered cuttings and its abundant sporulation on the cankered stubs on stock plants. *Phomopsis* sp. were not found on stock plants.

Isolates from cankers varied in cultural characteristics and pathogenicity; variation among isolates of *C. chrysosperma* has been noted (5,11).

Isolations from and incubation of the two poplar cultivars indicated that Norway are more resistant than Northwest poplars to canker fungi. This differential susceptibility to canker fungi among cultivars has been noted by others (2–4,7). The difference was correlated with field observations in 1978, when the percentage of salable plants was higher with Norway than with Northwest poplars. Comparable information was not available for 1978–1979 stock.

Results of fungicide treatments in laboratory isolations and incubations differed from field trials. Fall-sprayed cuttings developed more severe cankers in the laboratory than did untreated controls, but in the field more salable plants were produced by the fall-sprayed cuttings than by controls.

C. chrysosperma can become established before or during cold storage. In a warm environment, the fungus can develop rapidly while the cutting comes out of dormancy. A fungicide application that prevents establishment of *C. chrysosperma*, at least until after cuttings are planted, increased production of salable plants. In our experiments, a fall dip treatment was best. If all cuttings during 1978 and 1979 had been dip treated in the fall and growth was similar, poplar production at this nursery would have increased by more than 50%.

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