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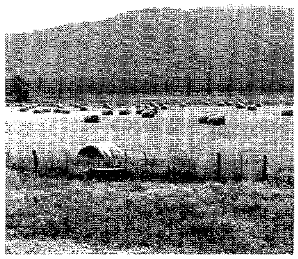
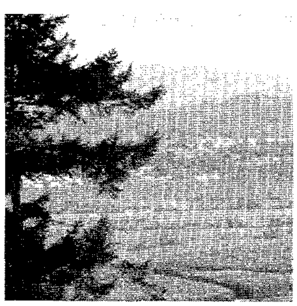
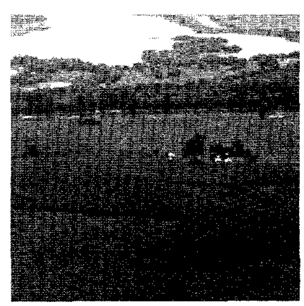


Short Rotation Woody Crop Trials for Energy Production in North Central U.S.





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Short Rotation Woody Crop Trials for Energy Production in North Central U.S.

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ABSTRACT

Tree plantations at several sites have numerous clones with heights greater than 45 feet and diameters of 6+ inches in eight years. A number of clones in associated small-plot hybrid trials have even better growth than the commercially available clones planted in large blocks at the same sites. The fastest growth rates have been attained in a plantation on a wet site at Milaca, MN, a plantation at Granite Falls, WI, and a plantation at Mondovi, WI, where the largest trees are up to 8 inches DBH at age 8. Mean annual production averages 3 dry tons per acre per year for the 5 best clones over all the test sites, and is still increasing. Mean annual production ranges from 4 to 5+ dry tons per acre in the best clonal blocks, and up to 8.1 tons per acre for the best new hybrids being tested in small-plot trials. The small-plot hybrid trial data indicate potential future biomass increases as new clones are introduced into commercial plantings. Reduced growth at some sites was related primarily to insufficient soil water during the growing season, and susceptibility to the disease Septoria musiva. Most tree mortality (36 percent) occurred during the establishment year with only an additional 2 percent mortality over the next 7 years. Leaf tissue nitrogen (N) levels decreased as trees aged and approached the hypothesized 3 percent critical level as trees reached 5- and 6-years old. Fertilization at 75 and 150 lbs/acre N resulted in significant increases in leaf tissue N both in extensive trials replicated across the plantation network and in an designed fertilizer trial at Milaca that was replicated over years. However, no significant increase in tree growth has been detected. There are significant clonal differences in leaf tissue nitrogen. Hybrid poplar plantations planted on agricultural fields produce significant increases in soil carbon, although there may be carbon loss during the early years of plantation establishment. Septoria musiva is the major pathogen affecting survival and growth of hybrid poplar plantations. A collection of 859 Septoria musiva and Septoria populicola isolates has shown considerable variability in the microorganism. Inoculation tests indicate that host specificity may need to be considered when screening clones for resistance. Tissue culture techniques are being used to increase resistance to Septoria in clone NE-308. The tissue culture procedure has been optimized for that clone and over 200 generation "2" plants are ready for field testing in 1995.

INTRODUCTION

Objectives:

The primary objective of this energy plantation program is to acquire information essential for establishing commercial short rotation fuelwood plantations in the north central region of the U.S. The focus of this effort is a plantation network across the north central states designed to provide improved measures of potential commercial biomass yield in this region, and to determine costs of wood energy from short-rotation woody crop (SRWC) plantations. The establishment of these large-size SRWC plantations in cooperation with an industrial user facilitates transfer of research expertise to the private sector and permits identification of operational problems that may require further research.

The objective of the biomass component is to determine commercially attainable biomass yields given the best site tending possible under the constraints of this extensive plantation network. Biomass yields were obtained for the current best clones at each of 8 sites to identify clones and sites that have the greatest yield. Analysis of the entire data set provides information on biomass yield potential across the region and provides clues regarding site parameters related to high yields.

Additional research objectives were incorporated into the program to advance the many elements of the program at about the same rate so that critical areas were not ignored. These additional areas of research have been underway for various periods of time and include identification of new faster-growing hybrids in clonal trials, developing improved weed control strategies, monitoring plantation nitrogen status with a leaf tissue bioassay, conducting nitrogen fertilization trials, determining if there is measurable soil carbon sequestration under older hybrid poplar plantations, developing a Septoria resistant "model" clone, and acquiring a better understanding of the Septoria pathogen for developing improved nursery screening techniques for new clonal material from the breeding program.

One of the primary limiting factors in the development of an efficient, reliable system using hybrid poplars for energy and fiber production is the high disease susceptibility of many clones that have been planted and tested. Chemical and cultural controls have been researched and tried operationally with varying degrees of success. Considering environmental variables, economics, and safety, genetic resistance is the most promising strategy to employ to minimize damage caused by disease. However, it must be remembered that the threat of disease outbreaks is always present owing to the ability of microorganisms to rapidly adapt to their hosts and evolve into more pathogenic strains. No matter what disease control strategies are used, constant vigilance against new pest populations, and a sustained effort in tree improvement will be needed to ensure continued success.

Hybrid poplars are subjected to many microorganisms which, under some environmental conditions, can cause disease to develop. Poplars are fast growing and have a wide geographic range, and are thus being considered for biomass plantings in many diverse areas. Microbe populations may be quite different from one region of the country to another, so poplar clones will need resistance to a large number of pathogens. Adding to this complexity is the presence of many species, races, biotypes, and strains of some genera of important poplar pathogens.

The two task activities related to protection funded under this agreement were to 1) examine the population of S. musiva to determine how variable this pathogen was in terms of causing disease and 2) apply somaclonal selection techniques to a selected poplar clone to increase resistance in that clone to S. musiva.

Background:

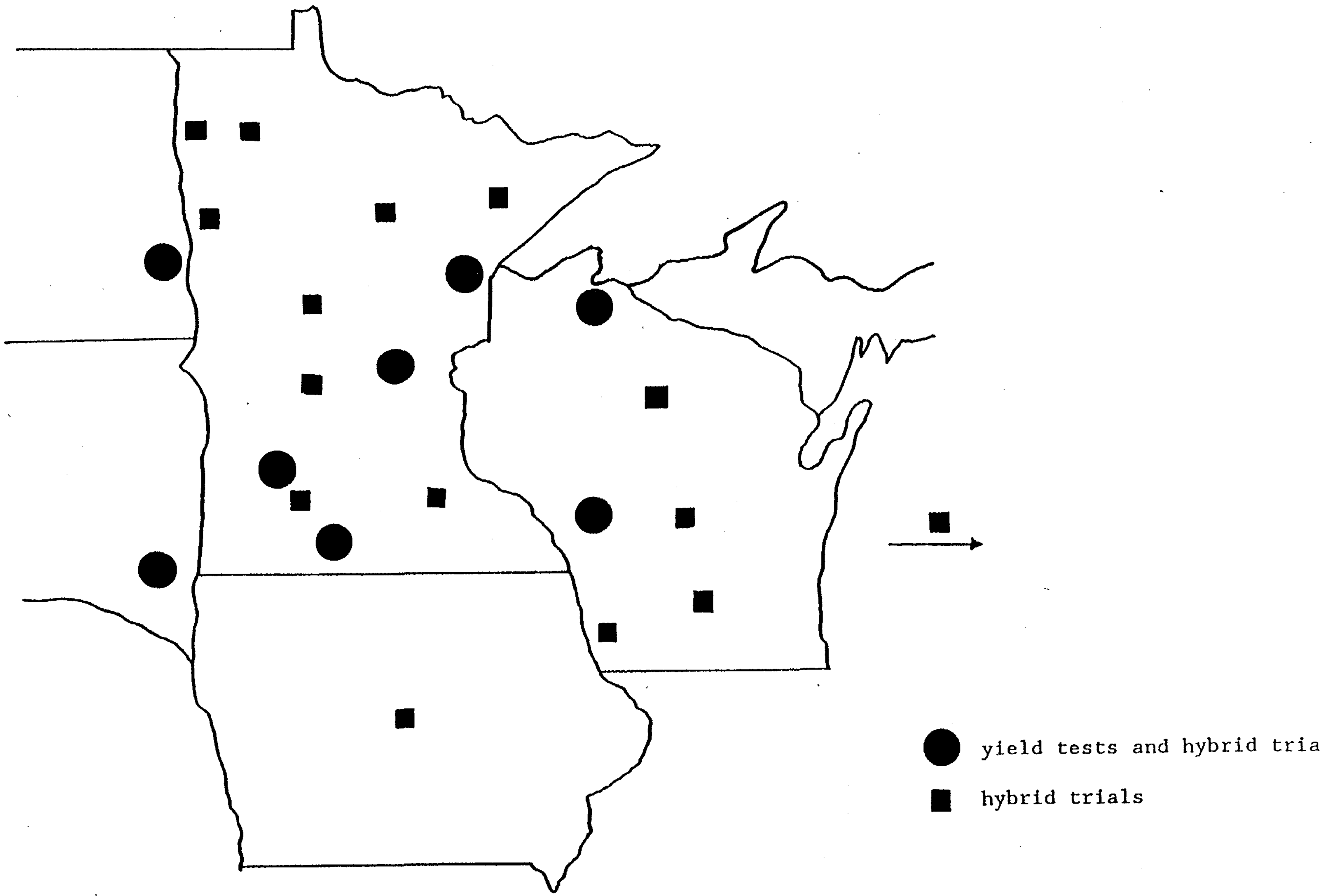
A network of research and demonstration energy plantations was established in 1987 and 1988 across a 4-state region in the north central U.S. (Figure 1). The primary criteria in site selection were: 1) the sites are within the region from the eastern edge of the Dakotas into western Wisconsin, 2) each site consisted of agricultural cropland with a single soil type, and 3) soil types were those occurring on large acreage in the region (see Table 1 for general site characteristics). This energy plantation network was cooperatively supported by the North Central Forest Experiment Station (USDA), the Oak Ridge National Laboratory (DOE), Energy Performance Systems Inc., and the Electric Power Research Institute. Approximately 130 acres out of the original planted 400 acres still remain in the program in 1995 despite loss of some sites from the historic drought in 1988 and 1989 and the loss of substantial acreage when some farmer participants withdrew from the program when changes in program sponsorship resulted in tardy land rent payments. Biomass production, tree survival, and data from supporting studies have been collected annually through the 1994 growing season and are summarized in this report.

Table 1. Description of Plantation Sites

State	County	Town	Soil Series	Texture	CER[1]	acres
MN	Martin	Fairmont	Coland	clay loam	62	10
MN	Chippewa	Granite Falls	Dupage	loam		15
MN	Mille Lacs	Milaca	Milaca	silt clay	42	14
MN	Carlton	Cloquet	Ahmeek	loam	42	10
ND	Cass	Fargo	Fargo	silt clay	67	20
SD	Minnehaha	Sioux Falls	Kranzburg	silt/c/loam	70	16
WI	Buffalo	Mondovi	Antigo	silt loam	50	16
WI	Ashland	Ashland	Ontonagon	silt clay	35	20

[1]Crop Equivalency Ratio

Figure 1. **Regional energy plantation network**



Plantation design:

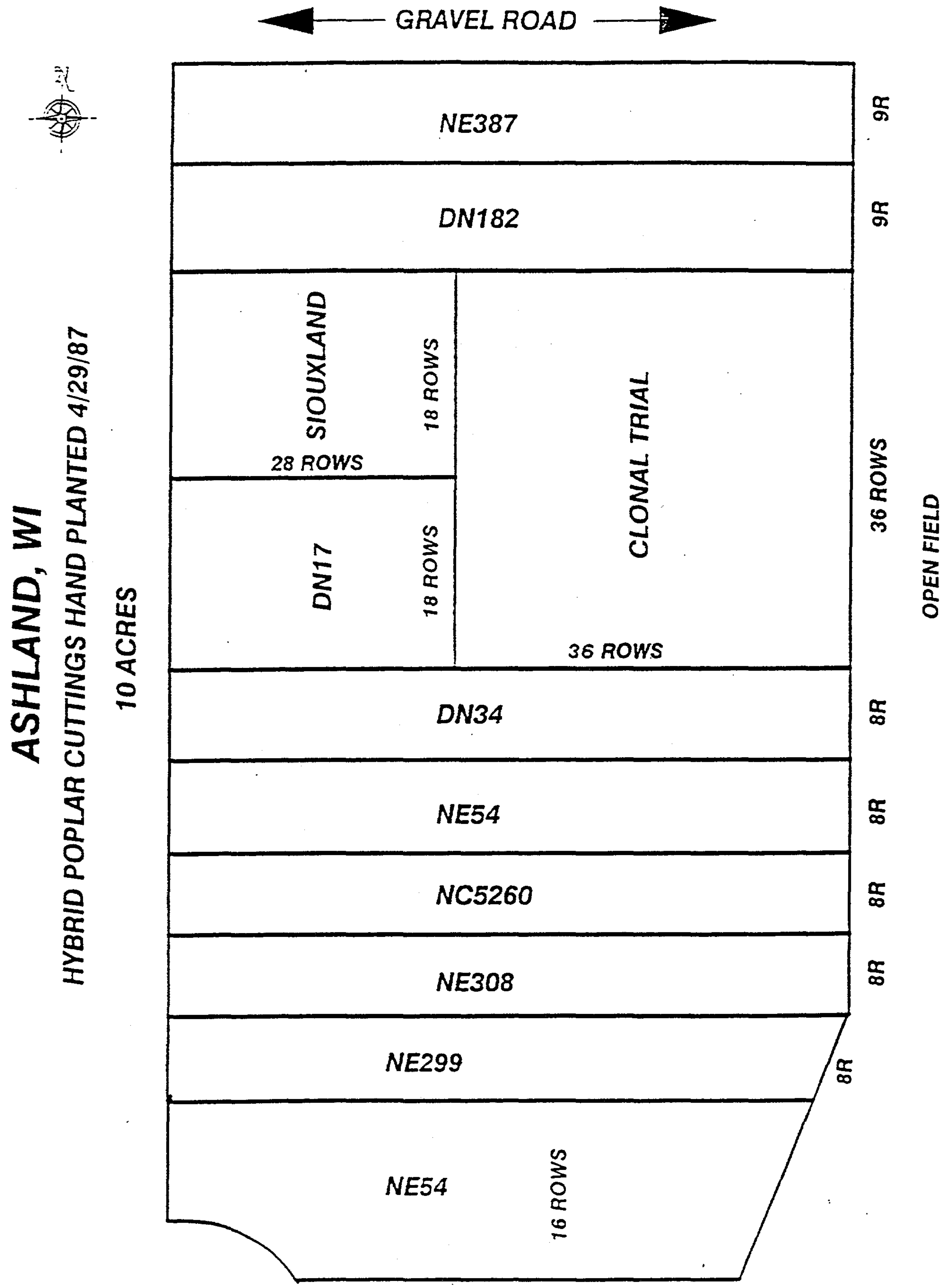
The basic planting design for the plantations is shown in Figure 2. Trees in all plantations were hand planted at an 8 x 8 foot spacing chosen to achieve maximum mean annual biomass production with an estimated rotation of 10 years. The 10-acre plantations were subdivided into 10 smaller monoclonal blocks with a different hybrid planted in each block to increase the probability of having one or more superior growing clones at each site. Each monoclonal block has an area of 0.8 acres. This block size is adequate to obtain valid biomass yield data, conduct soil-tree growth investigations, observe disease incidence, and eventually conduct harvesting trials. Three permanent 25-tree plots were established in each monoclonal block. Trees in these plots were remeasured annually to determine biomass production and tree mortality. Cultural operations on the entire 10-acre plantation were recorded to determine labor and cost inputs for evaluation of SRIC economics.

Hybrid trials were also planted using an identical planting design for all sites in a particular year, but a re-randomization of clonal plots each succeeding year. A different number of hybrids were planted each year; 79 in 1987, 67 in 1988, 49 in 1989, 42 in 1990, 56 in 1991, 44 in 1992, and 81 in 1993. Tentative selections of the best growing hybrids in the region are made each year to include in the following year's trial along with promising new material. These trials permit identification of better hybrids for future large-scale plantations.

Report format:

This report first summarizes results of the 1994 growing season, and then summarizes general findings over the eight-year period of investigation. Selected data sets that may be of use by others, but have not been previously published, are included in their entirety. Published subjects are briefly summarized and referenced to the publication. Other subjects that are unpublished, or have recently collected unpublished data are given a more detailed description of design, analysis and interpretation.

Figure 2. Planting design for typical 10-acre plantation



1994 RESULTS

Biomass:

Measurements of tree survival and dbh are collected each fall on the better clones at all sites. Periodically, selected trees are harvested, weighed green, subsampled and oven dried to determine total tree dry weight. Using these dried tree weight data, biomass regression equations of the form "Tree weight = a + b DBH^2" were developed to predict biomass production in the plantations. Equations were developed for each site for which there were sufficient trees to warrant doing so (n>20), which included Ashland, Mondovi, Granite Falls, and Milaca. For the remaining sites, a generalized regression equation developed from all the tree dry weight data from all sites (n=136) was used to calculate biomass production.

Biomass production in the 7- and 8-year-old plantations in the fall of 1994 ranged up to 5.3 dry tons/acre/year (TAY) (Table 2). Biomass yields of many plots are within the range first projected (3-6 TAY), and are still increasing through the 1994 growing season. Average biomass production for the two age-groups of plantation show 0.27 TAY (10%) more biomass in the younger 7-year-old plantations. The greater yields in the younger plantations are believed due to better survival during establishment, and to better first-year tending. Biomass production exceeds 4 TAY in six clonal block plantings; all of which are in seven-year-old plantations located at the two sites of Granite Falls and Mondovi (Table 2a). In addition, biomass production exceeds 3 TAY in many blocks scattered across most of the sites.

Table 2. Biomass production in 7- and 8-year-old plantations in fall of 1994 (actual standing biomass; oven-dry). Calculated from site-specific regression equations. X1 is the mean of specific clones (DN-17, DN-34, DN-182, NE-308 or Siouxland) over all sites; X2 is the mean of all those clones within a given site. (-) indicates biomass declined since 1993.

Table 2a. 7-year-old plantations.

CLONE	ASH	CLO	FAI	GRF	MON	SXF	$\bar{X}1$
	SITE (tons/acre/year)						
DN-1	*	*	*	*	*	*	
DN-17	1.7	0	2.8	4.6	3.0	2.0	2.82
NE-19	*	0	*	5.3	0	0	
DN-34	2.4-	2.1	3.4	4.1	4.7-	3.0	3.28
NE-54	1.9	0	0	-	0	-	
DN-182	2.3	2.3	3.5	4.0	4.2	2.3	3.10
NE-299	2.7	0	0	-	0	-	
NE-308	1.9	3.1	2.7-	3.7-	3.1	2.5	2.75
NE-387	1.9	0	0	-	0	-	
SIOUX	2.4-	0	2.4-	2.9	*	2.5-	2.55
NC-5260	0	0	0	-	2.7	0	
$\bar{X}2$ =	2.1	2.5	3.0	3.9	3.8	2.4	2.95

Table 2b. 8-year-old plantations.

CLONE	ASH	FAR	GRF	MIL	MON	SXF	$\bar{X}1$
	SITE (tons/acre/year)						
DN-1	*	*	3.0	*	*	*	
DN-17	2.3	3.2	3.6	3.9	3.8-	2.6	3.23
NE-19	*	0	*	*	*	*	
DN-34	1.1	0	3.7	2.6	3.3	1.8	2.50
NE-41	*	*	*	0	*	0	
NE-47	*	*	*	*	0	*	
NE-54	0	0	0	0	0	0	
DN-182	1.3	3.0	+	3.5	3.9-	2.4	2.82
NE-299	2.4	0	0	0	0	0	
NE-308	2.0-	0	2.7	3.1	2.4-	2.8	2.60
NE-387	1.6	0	+	0	0	0	
SIOUX	0.9	2.6	2.3	3.1	2.7	1.5	2.18
NC-5260	1.2	0	1.9	2.2	2.1	1.8	
$\bar{X}2$ =	1.5	2.9	3.1	3.2	3.2	2.2	2.68

* Clone not planted.
0 Measurements terminated because of poor growth.
\+ Clone removed for road construction.

Fertilization trials:

Beginning in 1990, leaf samples were collected annually in early July at each site from three clones (DN17, DN34, and DN182), and analyzed for nitrogen (N) concentration. In 1992 and 1993, following leaf sample collection, one of the three biomass measurement plots in each clonal block was randomly selected and fertilized with 150 lbs nitrogen (different plots each year), leaf samples collected again 3 weeks later from both fertilized and unfertilized plots, and then annually thereafter. Tests were made by ANOVA for significant differences in leaf nitrogen between the fertilized plots and the unfertilized control plots.

Plots throughout the region (both fertilized and unfertilized), showed much lower leaf N in 1994 as compared to 1993. Site averages ranged from only 2.3 - 2.9% in 1994 vs 3.0 -3.8% in 1993 (exceptions were Fargo at 2.3% in 1993 and Fairmont at 3.3% in 1994). Leaf N in 1994 was significantly higher (p=0.02) from plots fertilized in 1993 than from the unfertilized control plots (Table 3). However, there was no significant difference in 1994 leaf N between 1992 fertilized and control plots. These results suggest that the fertilization effect of increased leaf N lasts only one year.

Fertilization did not result in increased tree growth as measured at DBH. Comparison of changes in mean tree diameter between fertilized and unfertilized plots showed no significant differences when analyzed by regression for either the 1992-1993 or the 1993-1994 growing period for the 1992 fertilization, the 1993-1994 growing period for the 1993 fertilization, or for the entire period of record (1991-1994) for either fertilization date.

A replicated fertilizer trial was conducted at Milaca, one of the sites shown in Figure 1. Three of 9 replicated plots were randomly selected in 1992 (plantation age was 4 years old) and treatments of 0, 75, and 150 lbs/acre N applied. Three more plots were selected randomly in 1993 and 1994 and the treatments repeated. Both the 75 and 150 lbs/acre of N fertilization treatments in 1994 (below) showed significant increases in leaf N relative to the control plots.

	pre-fertilization	post-fertilization
	---------- plot means (%N)	-----------
control	2.4	2.7
75 lbs N	2.3	3.5
150 lbs N	2.3	3.6

The data are the average of 3 leaf samples from each of 12 trees per plot. There are 12 clones per plot, one tree of each clone. A regression analysis of treatment effect using individual trees as the sample (Leaf N ~ treatment + clone) showed a borderline significant difference between treatments PRIOR TO nitrogen fertilization (p=0.049), but a highly significant differences between treatments following fertilization (p=0.000). Also, an analysis of the CHANGE IN

Table 3. Leaf N concentration as influenced by year of fertilization.

Site	Clone	1994 Leaf N Concentration Control	Fert 1992	Fert 1993
Ashland	DN17	2.43	2.26	--
	DN34	2.19	2.41	2.41
	DN18	2.19	2.35	2.42
Cloquet	DN34	2.35	2.85	2.85
	DN182	2.20	2.72	2.97
Fairmont	DN17	3.11	3.15	3.34
	DN34	3.30	3.13	3.51
	DN182	3.57	3.45	3.34
Fargo	DN17	2.63	1.88	--
	DN182	2.20	2.74	--
Granite Falls	DN17	2.94	2.84	--
	DN34	2.78	2.91	--
Milaca	DN17	2.37	2.68	2.29
	DN34	2.55	2.50	--
	DN182	2.35	2.23	2.78
Mondovi	DN17	3.07*	2.94	3.13
	DN34	3.10*	2.64	--
	DN182	2.49*	2.88	--
Sioux Falls	DN17	2.79	2.90	2.83
	DN34	2.77	2.87	2.91
	DN182	2.54	2.57	2.72
	$\bar{X}$ =	2.68	2.68	2.88**

* Control data are relatively high because the entire plantation was fertilized in August 1991.

** Significantly different from the unfertilized controls leaf N (pretreatment - post-treatment) showed a highly significant difference between treatments (p=0.000). However, these analyses are weak because there is no randomization of treatments with this single year analysis. The results of a multi-year analysis (with replication and randomization) will be presented later in the summary section.

The effect of nitrogen fertilization on tree diameter was mixed. Based on an analysis with tree as the sample (no randomization), the plots receiving the 1992 fertilization treatment showed no significant growth response in either 1992 or 1993. On the other hand, the 1993 fertilization treatment showed a highly significant effect in 1994. However, this latter result is suspect since the control plot was lagging progressively behind the treatment plots even before the treatment was applied. A more complete analysis involving the entire data set is described in the summary section.

Clonal performance:

Analysis of clonal effect on leaf N in the Milaca test was done by pooling all treatments into pre- and post-fertilization groups. Results showed that there was a highly significant difference between clones in leaf N prior to fertilization, but no significant difference after fertilization. This suggests that leaf N concentration differs between clones under limiting nutrient conditions, but tends to be similar between clones under conditions of luxury consumption.

Analysis of clonal growth differences in the Milaca test (all treatments pooled) shows highly significant differences as follows:

Clone	Dbh (mm)	
NM6	143	a **
DN131	122	b
I45/51	117	b
DN55	100	c
*DN182	98	c
*SIOUX	98	c
*NE308	92	cd
*DN34	88	cde
*DN17	88	cde
NE20	76	def
NE387	73	ef
NC5260	71	f

* Clones in regional monoclonal block trials.
** Clones followed by same letter are not significantly different.

Note that one clone is performing as well, and three clones are performing significantly better than the five best clones in the monoclonal block trials (the present commercially available clones in the region).

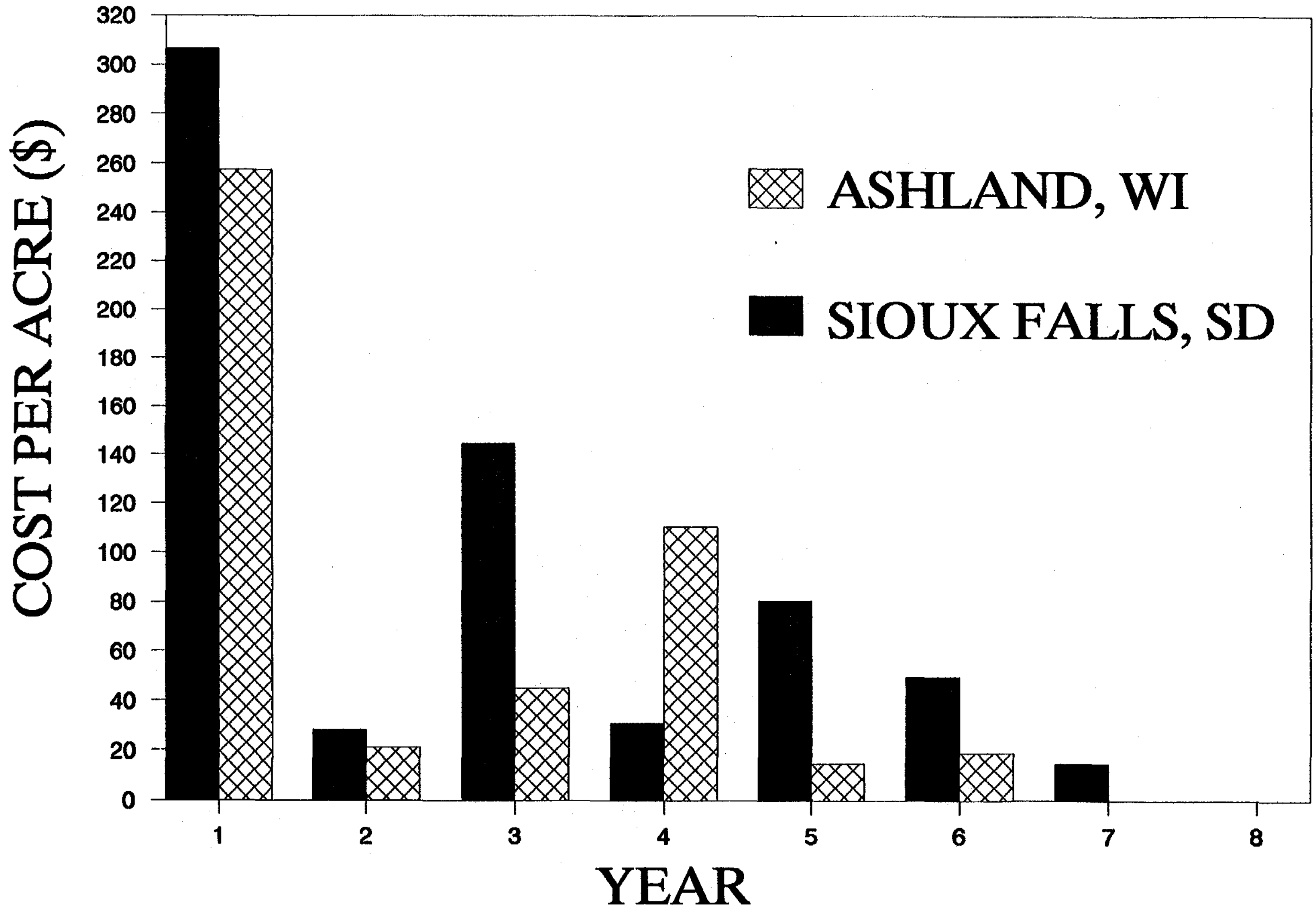
SUMMARY FOR 1989-1994

Plantation maintenance labor and cost:

Detailed cost records were kept for two sites in the regional trial network (Appendix A). The sites were Ashland, WI, and Sioux Falls, SD. Ashland is one of the northern sites in a marginal farming area in northwestern Wisconsin. Sioux Falls is one of the southern sites located on productive farmland in eastern South Dakota that has a cropping history of small grain and corn production. Each site has weed problems unique to the location which require different management strategies. At Ashland perennial grasses, mainly quackgrass (Agropyron repens, L.), were most common. These perennial grasses were controlled with grass herbicides such as fluazifop during the growing season or glyphosate when the trees were dormant. At Sioux Falls, on the other hand, broadleaf annual weeds such as redroot pigweed (Amaranthus retroflexus, L.) were the main weeds competing with the hybrid poplar. Mechanical cultivation was used to control these weeds since herbicides for broadleaf weed control also damage the trees. In some cases where mechanical cultivators could not get close enough to the trees without damaging the roots, the weeds were controlled by hand weeding. In addition, tractor access into the plantation was more limited at Ashland due to the clay soils which prohibited tillage during wet periods.

Costs through year eight are illustrated in Figure 3. At Sioux Falls total cost was $656.10 per acre compared with a total cost per acre at Ashland of $468.98. Establishment and tending costs in the first year represent approximately one-half of the total costs for each site. Equipment and labor costs at Sioux Falls were $358.70 and materials cost $300.40. This compares to equipment and labor costs of $201.54 and materials cost of $267.44 at Ashland. At Sioux Falls materials cost less than labor and equipment, reflecting the greater availability of chemicals and other farm materials in this agricultural area. Ashland, on the other hand had a higher cost for materials compared to labor reflecting the difficulty in obtaining farm chemicals in this marginal farming area. In addition, the more persistent weeds especially from broadleaf weeds at Sioux Falls required greater use of mechanical and hand labor for control. Noxious weed control, especially thistle, also required additional efforts to control. Actual labor costs were recorded for all contract operations. When the operations were performed by USDA staff the recorded cost was obtained from the custom rate guides published by state agencies for agricultural operations. The cost of chemical weed control (simazine) in year three at Sioux Falls and year four at Ashland will likely be greatly reduced in future plantations. Cost of simazine was approximately ten times higher than the chemicals such as sulfometuron that will likely replace it. There were no tending costs in year eight. Cost of operations discussed here are typical of the costs likely incurred through year three or four with the exception of the cost of simazine. Costs beyond year four will likely not be

FIGURE 3 HYBRID POPLAR PLANTATION ESTABLISHMENT AND TENDING COSTS BY YEAR.



necessary in commercial operations unless noxious weed control is required by local ordnance. The best clones selected from this research, when planted in large blocks, will have canopy closure sooner than these test plantations that contained both good and poor clones. Earlier canopy closure will result in reduced costs over those reported here.

Biomass prediction equations

Previously reported biomass estimates were based on a prediction equation of the form "Log_e tree dry weight = a + b Log_e DBH". This equation fit reasonably well until 1994. With the addition of tree dry weight data for larger trees, this equation form under-predicted the dry weight of larger sized trees, resulting in an under-estimate of biomass on some sites. Consequently, different equation forms were tried, and the form "tree dry weight = a + b DBH^2" was selected as a good predictor and with the residuals showing little bias. Tree diameters and weights for the dry-weight data set ranged from 1- to 20-cm and .5- to 93-kg respectively (Appendix A2).

Regression equations were derived for each site and clone (Table 4). Although there were no significant differences in variance or slope between equations, the Milaca site regression tended to differ slightly from the other sites (trees weighed less for a given dbh). Including the Milaca tree dry weight data in a pooled data set resulted in lowering the biomass estimates for all sites, except Milaca which was increased. Consequently, site-specific biomass regression equations were derived for all sites for which there were sufficient tree dry weight data. These site-specific regressions resulted in higher biomass estimates for Ashland, Granite Falls, Mondovi and lower estimates for Milaca. Biomass estimates for the remaining sites were calculated using the generalized regression equation (all tree dry weight data pooled). Use of the site specific equations as compared to the generalized regression equation resulted in an overall increase in biomass prediction of 3 percent for the plantation network, and a maximum of 8 percent for a single site (Granite Falls 1988 planting). Milaca declined 6 percent.

Since the new site-specific equations based on DBH^2 fit the data significantly better, and no trees had been harvested in 1993 with which to modify the equations, the newly derived equations were used to recalculate the 1993 biomass estimates. These revised estimates are shown in Table 5. Biomass calculated with the new equations increased 23 percent for the 6-year-old plantations, and 13 percent for the 7-year-old plantations over that reported in the 1993 DOE Annual Report.

Another possible source of error in addition to the goodness of fit of the regression equations, is the accuracy at which the subsampling procedure provides an unbiased estimate of the total tree dry weight.

A limited test was conducted on one tree with a dbh of 16.7 cm, where in addition to subsampling in the usual manner, the entire tree was dried and weighed. The subsample over-estimated total tree dry weight by 1.5 percent.

Table 4. Regression equation coefficients for the North Central U.S. plantation network. (Tree weight = $a + bDBH^2$)

Equation	a	b	r^2	n
General equation	-1.67	.231	98.5	136
by site:				
Ashland, WI	.60	.208	98.5	34
Mondovi, WI	-3.56	.255	99.0	27
Granite Falls, MN	-2.12	.249	99.7	24
Milaca, MN	-3.16	.226	97.1	24
Milaca, MN (NE clones)	-.04	.206	93.5	13
Fairmont, MN	.96	.199	92.7	8
by clone:				
DN34	-2.22	.243	98.6	34
DN17	-1.10	.230	98.2	34
DN182	-1.08	.223	98.1	25
NE308	-.51	.190	91.3	21
NE54/NE387	-.04	.206	93.5	13
Siouxland	.96	.199	92.7	8

Table 5. Biomass production in 6- and 7-year-old plantations in fall of 1993 (actual standing biomass; oven-dry). Calculated from site-specific regression equations. $\overline{X}1$ is the mean of specific clones (DN-17, DN-34, DN-182, NE-308 or Siouxland) across all sites, and $\overline{X}2$ is the mean of all those clones within a given site.

Table 5a. 6-year-old plantations.

CLONE	SITE: ASH	CLO	FAI	GRF	MON	SXF	$\overline{X}1$
	tons/acre/year						
DN-1	*	*	*	*	*	*	
DN-17	1.6	0	2.7	4.5	3.0	1.8	2.72
NE-19	*	0	*	5.2	0	0	
DN-34	2.5	1.7	3.2	3.9	4.8	2.8	3.15
NE-54	1.6	0	0	0	0	0	
DN-182	2.3	2.0	3.5	3.8	4.2	2.2	3.00
NE-299	2.4	0	0	0	0	0	
NE-308	1.9	2.7	3.0	3.9	3.1	1.8	2.73
NE-387	0	0	0	0	0	0	
SIOUX	2.5	0	2.5	2.8	*	2.6	2.60
NC-5260	0	0	0	0	2.6	0	
$\overline{X}2$ =	2.2	2.1	3.0	3.8	3.8	2.2	2.84

Table 5b. 7-year-old plantations.

CLONE	SITE: ASH	FAR	GRF	MIL	MON	SXF	$\overline{X}1$
	tons/acre/year						
DN-1	*	*	2.8	*	*	*	
DN-17	2.2	2.9	3.4	3.6	4.1	2.4	3.10
NE-19	*	0	*	*	*	*	
DN-34	1.0	0	3.4	2.2	3.3	1.5	2.28
NE-41	*	*	*	0	*	0	
NE-47	*	*	*	*	0	*	
NE-54	0	0	0	0	0	0	
DN-182	1.3	2.8	+	3.2	4.1	2.1	2.70
NE-299	2.3	0	0	0	0	0	
NE-308	2.1	0	2.5	3.0	2.6	2.7	2.58
NE-387	1.6	0	+	0	0	0	
SIOUX	0.9	2.3	2.1	2.8	2.7	1.3	2.02
NC-5260	1.2	0	1.8	2.0	2.0	1.8	
$\overline{X}2$ =	1.5	2.7	2.8	3.0	3.4	2.0	2.56

* Clone not planted.
0 Measurements terminated because of poor growth.
\+ Clone removed for road construction.

Biomass production:

Mean annual biomass production increased at most sites through 1994 (years 7 and 8) (Table 6). Only the Mondovi plantations are declining in production, and the oldest plantation at Ashland is static. The declines at Mondovi may be due to several possible causes including chemical damage (DN-34, 1988), Septoria musiva (DN-17 & NE-308, 1987), and physiological maturity (DN-182, 1987). A few clonal blocks at most of the sites declined in biomass between 1993 and 1994 (Table 2). The frequency of decline was related to clonal disease resistance. DN-17, DN34, and DN182 which are some of the more resistant clones declined in only 1 or 2 blocks across the region (both plantation ages pooled). Whereas NE-308 and Siouxland clones which have a higher incidence of Septoria infection declined in 3 or 4 blocks.

Overall, biomass increased between 1993 and 1994 from 2.84 to 2.95 tons/acre/year (TAY) in the younger plantations, and from 2.56 to 2.68 TAY in the older plantations (Tables 2 and 5). For both ages the increase was between 4 and 5 percent. The original projections were that the plantations' mean annual biomass production would peak at age 10 for the 8 x 8 foot tree spacing. In view of the trends in Table 6, it appears that this is still a reasonable projection.
Recommendation: CONTINUE ANNUAL BIOMASS MEASUREMENTS UNTIL BIOMASS PRODUCTION HAS PEAKED AT MOST SITES.

The question of greatest interest is "what is the likely biomass yield in the region?" There are a number of clues to this question. Biomass production of the five best clones planted over all sites, currently averages nearly 3 TAY (Table 2). The best clone (DN-34) over all sites averages 3.3 TAY, and the best site (Granite Falls) averages 3.9 TAY over all clones. The combination of the best clone x site produces yields of 4.6-4.7 TAY. (The yield of 5.3 TAY for clone NE-19 at Granite Falls is discounted since the clone did so poorly everywhere else). So, with these five clones, one can expect yields of about 3 TAY with minimal site selection and 3.9 TAY with site selection. With site selection and using the current best clones, yields of 4.7 TAY are achievable. Keeping in mind that yields have not yet peaked in these plantations, these estimates are expected to increase somewhat in the next few years. Introduction of more disease resistant and faster growing clones will increase the yield potential over that reported here. Yields of some clones in the hybrid trials range up to 8.1 TAY illustrating the potential for greater yields with better clones. Also, reduction of early tree mortality (discussed in next section) would result in some increase in biomass.

This type of analysis does not address the issue of what percent of a large scale planting operation will actually be on high quality sites. Characterization and identification of good sites is poorly developed at this time. Some, (perhaps many) sites in a large planting program may be of poor quality. The effect of variable site quality (and consequently rigorous site selection) on average yields is illustrated by the data for 7-year-old plantations in Table 2 where the yield of the poorest and best site was 2.1 and 3.9 TAY respectively, and the average yield across all sites was right in the middle with 3.0 TAY.

Table 6. Mean Annual Biomass Production by site and year of planting. Data are the mean of the three best clones (DN17, DN34, and DN182).

Site	Year					
	1989	1990	1991	1992	1993	1994
	tons/acre/year					
1987 plantings:						
Ashland	.15		.97	1.27	1.50	1.57
Fargo	.29		1.85	1.75	2.85	3.20
Granite Falls	.79		1.63	1.90	3.40	3.65
Milaca	.75	1.55	2.10	2.53	3.00	3.30
Mondovi	.55	1.82	2.53	3.23	3.83	3.67*
Sioux Falls	.59		1.10	1.73	2.00	2.27
1988 plantings:						
Ashland			1.67	1.93	2.13	2.13*
Cloquet			1.25	1.75	1.85	2.20
Fairmont			1.93	2.27	3.13	3.23
Granite Falls			1.93	2.33	4.07	4.23
Mondovi		2.05	2.73	3.50	4.00	3.97*
Sioux Falls			1.23	1.60	2.27	2.43

* Biomass production is static or declining from 1993 to 1994.

Tree mortality:

There was substantial tree mortality during the establishment year due to inadequate site preparation and weed control, the extensive nature of the plantation network, and to a lesser extent a historic record drought. Tree survival at the end of the first year averaged 73 percent. Since then, there has been little mortality of the better clones. (Some clones susceptible to disease had mortality of 100 percent at a young age.) Survival of the five best clones averaged 70 percent at the end of the first year across four sites (Table 7). Additional mortality through the eighth growing season was only 5 percent. Clone NE-308 was unusual in that it had high initial survival but higher than average mortality in later years due to its susceptibility to Septoria. Deleting the NE-308 data in Table 7 results in an average first year mortality for the remaining clones of 36 percent followed by only 2 percent additional mortality over the next seven years. With good site preparation and weed control, survival in individual clonal plots ranged up to 98 percent. The conclusion is that establishment year cultural practices are extremely important for initial survival, and that ensuing mortality in well-established and tended plantations is negligible.

Table 7. Tree survival at the end of year 1 and year 8 for selected sites and clones.

Clone	Ashland year 1	Ashland year 8	Granite Falls year 1	Granite Falls year 8	Milaca year 1	Milaca year 8	Sioux Falls year 1	Sioux Falls year 8
	% survival							
DN17	92	90	87	82	80	79	80	79
DN34	41	37	64	64*	42	40	37	35
DN182	44	41	--	--	63	60	76	75
NE308	83	76	80	56	91	84	87	81
Siouxland	52	50	72	68	72	75	61	57
$\bar{X}$ =	62	59	76	68	70	68	70	65

* Plot moved due to removal of original plot from road construction.

Tree mortality and biomass production:

A question that arises is "what would the biomass production be if survival had been 100 percent"? Data in Table 6 show that the 7-year-old (younger) plantations have greater biomass. At least part of this greater yield is due to greater survival (63 vs 58 percent for the 7- and 8-year-old plantations respectively). Therefore, there is some basis to argue for adjusting yields based on survival. The major problem in making these estimates is that when trees die, the remaining living trees grow faster because they have additional growing space. This is called "compensatory growth". If the mean tree size of living trees is then used to calculate total biomass production assuming all trees had lived, the result is an over-estimate of the biomass. The size of this bias cannot be calculated with the data at hand. But the following illustrates that it exists: Biomass production in the measurement plots was significantly (positively) related to percent tree survival at many sites in 1991 and 1993, but at only one site in 1994 (Ashland--the slowest growing site, see Table 6). The declining frequency of significance over time suggests that trees in the lower-stocked plots (greater mortality) are growing faster and their biomass yields are catching up to the better-stocked plots. Recognizing that there is a bias to the procedure, the following calculations still provide a useful "upper-bounds" on biomass production estimates for the clones, site, and cultural tending under consideration. They do not necessarily represent achievable yields with 100 percent survival.

Projected biomass yields at 100 percent survival were calculated in two ways. First, the mean plot biomass for a site was adjusted by its difference in survival from 100 percent. Therefore, if the measured mean plot survival were 50 percent, the measured biomass would be doubled to give the resultant "adjusted mean site yield" at 100 percent as shown in Table 8. This gives an estimate of what the mean site yield might be for the mix of clones planted if survival were 100 percent. In the case of Ashland and Sioux Falls that already had high survival, this procedure produced little increase over the measured yields. On the other hand for Milaca and Mondovi where tree survival was lower, projected yields increased somewhat more. Since this procedure includes poor clones along with good clones, and includes areas of clumped mortality along with individual missing trees, it contains factors that tend to offset the bias originating from compensatory growth. It is our judgement that yields with 100 percent survival with this set of clones, sites, and cultural tending would probably fall somewhere between the "current" and "adjusted" values for the "mean site yield" in Table 8.

A second procedure was to adjust the plot with the maximum biomass on each site by the difference of its measured survival from 100 percent survival. This procedure is judged to give estimates biased high because it is based on the record yield plot for each site which may not reflect the average potential for the entire site, and makes no allowance for compensatory growth.

Table 8. Calculated site and plot biomass yields adjusted to a theoretical 100 percent survival (projected yields).

Site	mean site survival*	mean site yield current	adjusted	high plot yield current	adjusted
	-- % ---	---------- ton/acre/year			--------------
Ashland	82	2.2	2.6	2.7	2.7
Cloquet	76	2.5	3.3	3.1	3.9
Fairmont	78	3.0	3.8	3.5	4.3
Fargo	73	2.9	4.0	3.2	4.3
Granite Falls	76	4.1	5.4	5.3	6.6
Milaca	68	3.1	4.5	3.9	4.9
Mondovi	69	3.8	5.5	4.7	6.1
Sioux Falls	89	2.4	2.7	3.0	3.0

* Survival data does not generally correspond to data in Table 6 since Table 8 data are primarily from 7-year-old plantations.

Fertilization trials: (effect on leaf N)

Operational fertilization of the regional plantation sites was based on guidelines from nutrient cycling/fertilization trials conducted earlier at Rhinelander applied in conjunction with systematic leaf sampling of the plantations during the mid-growing season. Based on previous research, it was believed that N is the nutrient most likely to be deficient and it was hypothesized that when leaf tissue N concentration falls below 3%, there would be a growth response to fertilization. No fertilization was required during the first six years with the exception of potassium at Cloquet during the first growing season and N at Mondovi during the fifth growing season.

Annual monitoring of fertility status (with emphasis on N) began at all sites in 1990 when the plantations were in their third and fourth growing seasons. The objective was to determine when fertilization was needed, and to take appropriate action at that time. Leaf samples were collected from three clones (DN17, DN34, and DN182) at each site once during each growing season. In 1992 and 1993, leaf samples were collected in early July, randomly selected biomass measurement plots fertilized (different plots each year), leaf samples collected again from both fertilized and unfertilized plots 3 weeks later, and then at annual intervals. Tests were made for differences between the changes in nitrogen levels on the fertilized plots vs the changes in the unfertilized control plots for all sites pooled.

Fertilization in early July 1992 resulted in significant increases in leaf N as measured in the post-fertilization sample 3-weeks later. Leaf N increased 20 percent from 2.73 to 3.20 percent. Samples one year later (in 1993) showed that leaf N remained significantly elevated in the fertilized plots. However, two years after early July of 1993 showed the same pattern of significantly increased leaf N the first year following treatment (in 1994) (Table 3).

Similar results were obtained in the designed fertilizer test at Milaca. In that test, the same treatments were repeated on a new set of plots for three years in a row (Table 9). Each year was treated as a replication in the analysis. Since leaf N in the control plots generally increased over the 3-week pre/post sampling period, an ANOVA was done on the DIFFERENCES between the two sampling periods (post-pre) in leaf N. Both the 75 and 150 lbs/acre of N fertilization treatments over the three years showed a highly significant increase to N relative to the controls. Resampling the 1992 treatment plots in 1993 showed that leaf N was still increased over the controls, and by the same magnitude as in the year of fertilization. The data show a slightly higher leaf N with the 150 lbs/acre fertilization rate. But the difference over the 75 lbs/acre rate has no practical significance.

Table 9. Leaf nitrogen response in the year of fertilization at Milaca.

	1992		1993		1994	
	pre	post	pre	post	pre	post
	%N					
control	2.4	2.9	3.1	3.1	2.4	2.7
75 lbs N	2.4	3.3	2.8	3.5	2.3	3.5
150 lbs N	2.4	3.4	2.8	3.6	2.3	3.6

The results of the Milaca test corroborate the larger block plantings trials. They also agree with the previous single year analysis of Milaca results based on individual trees as the sample with no treatment randomization. These results show that fertilization at 75 or 150 lbs/acre N result in rapid increases in leaf N within 3 weeks which remain elevated for at least one year. One data set from the block plantings suggest that leaf N returns to the base level by the second year. Milaca measurements in 1994 were not made on the plots fertilized in 1992 and 1993, so this result on the duration of the fertilizer response could not be confirmed. <u>Recommendation:</u> MEASURE LEAF N ON ALL PLOTS IN THE MILACA FERTILIZATION TEST IN 1995 AND ANALYZE FOR DURATION OF FERTILIZER RESPONSE.

Fertilization trials: (effect on tree growth)

Measurements of the large block plantings showed no evidence of accelerated tree growth following fertilization in either 1992 or 1993. It is believed that one explanation for lack of any relation was the large variability in initial tree stocking. Poorly stocked tree plots grew faster than well stocked tree plots whether they were fertilized or not. Thus treatment was possibly obscured by variable tree stocking and hence variable growth rates.

There is some evidence from the Milaca test that fertilization may increase tree growth when measured in terms of annual dbh increment. The 1992 Milaca fertilizer treatment was followed by a 10 percent greater dbh increment in the fertilized plots (see replication 1 in Table 10). The 1993 treatment (replication 2) was followed by an even greater difference (29 percent) between the control and treated plots. However, the 1993 treatment data are suspect since the control plot has the smallest trees of any plot in the test and its growth had been progressively lagging behind the other plots even prior to treatment. Replication 3 was treated in 1994 and the results await collection of tree growth data in 1995. Analysis of the two replications (1993 replication 1, and 1994 replication 2) showed that the treatment effect is non-significant (p = 0.125). However it is quite possible that the addition of one more year of data (replication) will result in a significant effect of fertilization on tree growth.
<u>Recommendation:</u> MEASURE TREE DIAMETER ON ALL PLOTS IN THE MILACA FERTILIZER TEST IN 1995 AND ANALYZE FOR GROWTH RESPONSE TO FERTILIZATION.

Table 10. Tree growth (DBH) response in Milaca fertilization test.

Table 10a. DBH in mm.

Replication	Treatment	1991	1992	1993	1994
		DBH (mm)			
1	control	47.3	66.9	83.7	100.8
	75 lbs N	45.3	66.7	85.0	101.4
	150 lbs N	44.9	64.2	84.1	101.5
2	control	42.7	60.5	75.5	88.3
	75 lbs N	44.8	64.8	81.5	99.0
	150 lbs N	42.9	63.7	79.8	97.7
3	control	43.1	66.4	82.8	99.3
	75 lbs N	48.4	68.3	85.8	98.4
	150 lbs N	46.0	64.2	80.0	95.6

Table 10b. DBH increment by year.

Replication	Treatment	1992	1993	1994
		DBH (mm)		
1	control	19.6	{16.8}	17.1
	75 lbs N	21.4	{18.3}	16.4
	150 lbs N	19.3	{19.9}	17.4
2	control	17.8	15.0	{12.8}
	75 lbs N	20.0	16.7	{17.5}
	150 lbs N	20.8	16.1	{17.9}
3	control	23.3	16.4	16.5
	75 lbs N	19.9	17.5	12.6
	150 lbs N	18.2	15.8	15.6

{ } first year growth following fertilization treatment

Clonal performance:

Since 1986, 61 clonal trials have been established (41 remaining) with 40-80 clones in each trial (Hansen et. al, 1994). We tested more than 140 Populus selections in the program. An analysis of 91 clones planted in the 1987 and 1988 trials resulted in dropping 52 clones from the testing program because they did not exhibit reasonable growth or disease resistance on even half of the good sites. Plantations on drier (western) sites had fewer clones that met the selection criteria, and plantations on wetter (eastern) sites had more clones that met those criteria. Consequently test plantations were divided into "good" (eastern) and "harsh" (western) sites. More clones were judged reliable on good than on harsh sites, but the same clones that performed best on harsh sites were among those most reliable on good sites (Table 11). Nearly half of the 39 clones were 100 percent reliable on good sites. As site conditions (in this case primarily water availability) became less favorable, the number of reliable clones greatly decreased. Of the 39 clones, 25 clones on harsh sites did not meet the 50 percent reliability criterion in contrast to only 1 clone on good sites not meeting that criterion as determined by their frequency of being judged reliable on all planted sites.

The best survival and growth has been predominantly from clones of deltoides-nigra (DN) parentage. Other clones that are growing well are either nigra-maximowicsii, or pure deltoides parentage. Clones originating from the northeast U.S. (NE) are mostly severely diseased and some have died completely at some sites. The DN clones sold by regional commercial nurseries have been the best growers in our large block tests. These are DN-17, DN-34, and DN-182 (Robusta, Imperial Carolina or Eugenei, and Raverdeau respectively). However, there appear to be better clones under test in our hybrid trials. The best 36 clones from these trials were identified. These clones exhibit reasonable survival, good growth and moderate to high resistance to leaf rust and Septoria canker. These 36 clones were provided to a state nursery for scale-up of planting stock production. We have since rouged out about half of these clones and released six clones to commercial nurseries in spring 1993 (DN2, DN5, DN70, NM6, NE222, I45-51). These "newer" clones should be planted only on limited acreage until more experience is gained.

The results from the designed fertilizer test at Milaca permitted analyses of the 12 clones in the test. The results consistently showed highly significant differences between the clones in both leaf N and tree growth. The ranking of the clones in terms of growth corroborated the results of the larger hybrid trials across the region (see "1994 Clonal Performance"). Recommendation: CONTINUE ANNUAL GROWTH, SURVIVAL AND DISEASE ASSESSMENT OF THE HYBRID TRIALS UNTIL TRIALS WITH NEW GENETIC MATERIAL FROM ISU BREEDING PROGRAM HAVE MATERIAL AVAILABLE FOR COMMERCIAL RELEASE.

Table 11—*Ranking of 5- and 6-year-old clones* (planted 1987, 1988)*

Clone	Harsh sites Reliability	Harsh sites Sites	Good sites Reliability	Good sites Sites	
	Percent	*Number*	*Percent*	*Number*	
DN5	100	3	100	4	
NM6	100	3	100	4	
DN70	100	3	100	4	
DN2	84	6	100	8	
DN34	84	6	100	8	
I45-51	84	6	100	8	
DN17	66	6	100	8	
NE222	66	6	87.5	8	**
DN38	66	3	100	4	
DN177	66	3	100	4	
DN170	66	3	100	4	
I476	66	3	50	4	
DN128	66	3	0	4	
NE264	50	6	100	5	
DN9	33	6	87.5	8	
DN74	33	3	100	4	
NM2	33	3	100	4	
NC5377	33	3	100	4	
DN16	33	6	75	8	
45-1	33	6	75	8	
DN174	33	3	75	4	
DN131	33	6	62.5	8	
DN173	33	3	50	4	
DN179	33	3	50	4	
DN55	16	6	100	8	
DN182**	16	6	100	8	
DN1	0	3	100	4	
SIOUX**	16	6	87.5	8	
DN181	0	3	75	4	
NE35	16	6	62.5	8	
NE295	0	6	62.5	8	
DN18	16	6	50	8	
DN106	0	3	50	4	
NE49	0	6	50	8	
DN114	0	6	50	8	
NE300	0	3	50	4	
NC5339	0	3	50	4	
DTAC7	0	3	50	4	
DTAC26	0	3	50	4	

**Clones with >50 percent reliability on at least one of two clusters of sites, where "reliability" = the percent of sites on which a given clone exhibited good growth relative to the other clones and fair resistance to Septoria through age 5 or 6 years.*

***Clones in commercial production at this time (includes all clones above line).*

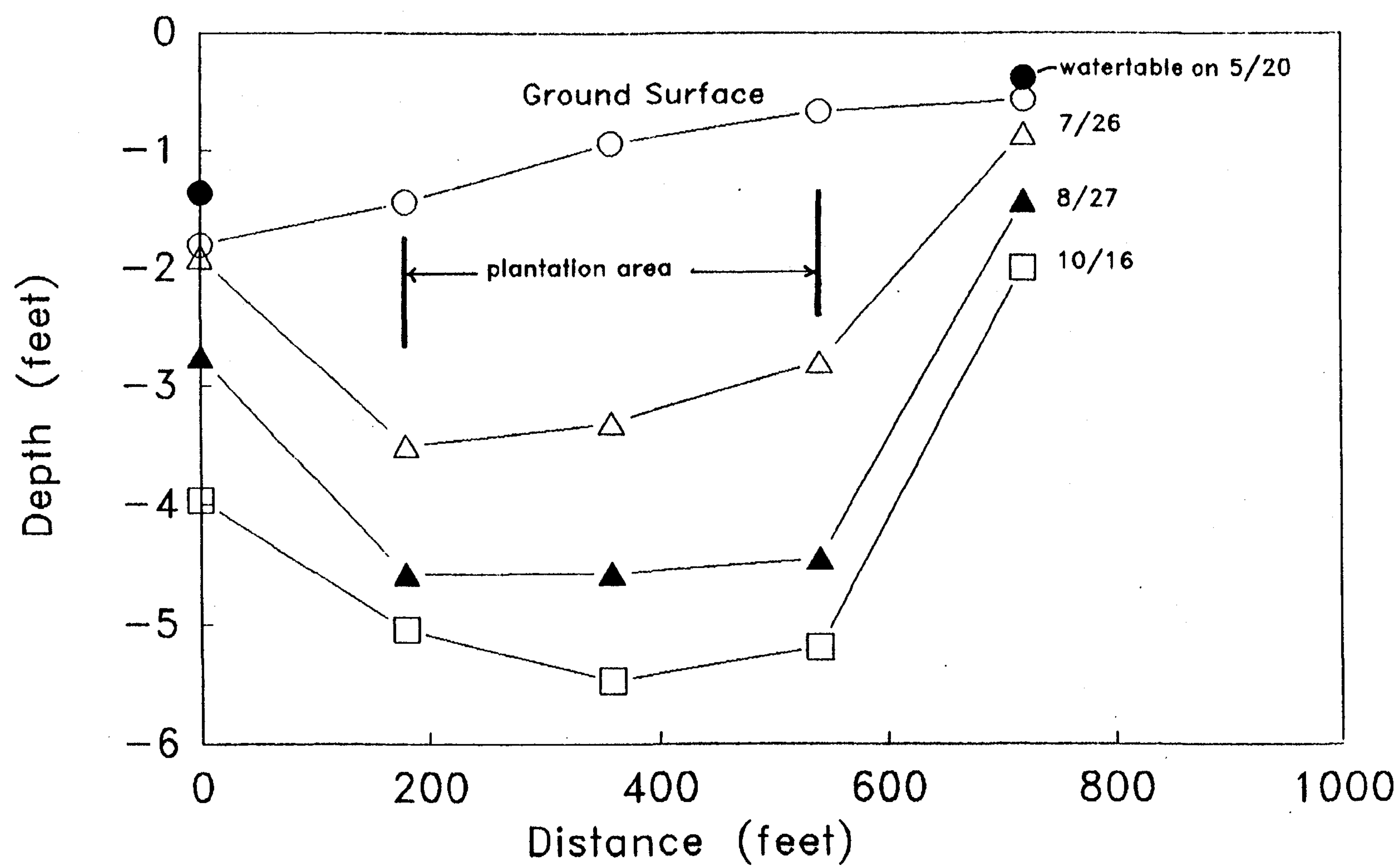
Soil water:

Measurements of total water use (consumptive use) have shown that hybrid poplar plantations use 1-2 inches more moisture a month than what is supplied by average growing season precipitation in central Wisconsin. A piezometer transect was established across the Milaca plantation. Measurements showed that the watertable (initially at the ground surface in May) declined beneath the plantation throughout the summer (Figure 4). The implication is that the trees were extracting much more water from groundwater than were the grass and shrub vegetation adjacent to the plantation. Since the Milaca plantation uses considerable groundwater in addition to rainfall input and is one of the fastest growing plantations in the network, it is concluded that water availability is a predominant factor in determining biomass production.

Soil Carbon:

This study investigated whether growing hybrid poplar plantations on tilled former grassland increases soil carbon (Hansen 1993). Existing hybrid poplar plantations were located across the region from eastern North Dakota into central Wisconsin. Nine sites were selected as suitable for the analysis. Comparisons were made in soil carbon levels between hybrid poplar plantations and adjacent row crops or mowed grass. It was concluded that hybrid poplar plantations grown on tilled agricultural lands previously in prairie, sequester significant quantities of soil carbon. Establishing and tending plantations often results in early soil carbon loss, but soil carbon is significantly related (positive) to tree age. Increasing tree age eventually results in a net addition of soil carbon from plantations older than about 6 to 12 years of age. Soil carbon loss under trees occurred most frequently from the surface 30 cm early in the plantation history--evidence that the loss was due to mineralization. Soil carbon gain was most significant in the 30-50 cm layer and was attributed to tree root growth. Soil carbon accretion rate beneath 12- to 18-year-old poplar plantations exceeded that of adjacent agricultural crops by 1.63 +- 0.16 Mg $ha^{-1}yr^{-1}$. There was a significant crop x soil depth interaction for bulk density with bulk density lower beneath trees in the 0-30 cm layer and higher in the 30-50 cm layer. There was little evidence of carbon trapping of wind-blown organic detritus by tree plantations in the prairie environment.

Figure 4. Watertable depression under the Milaca plantation.



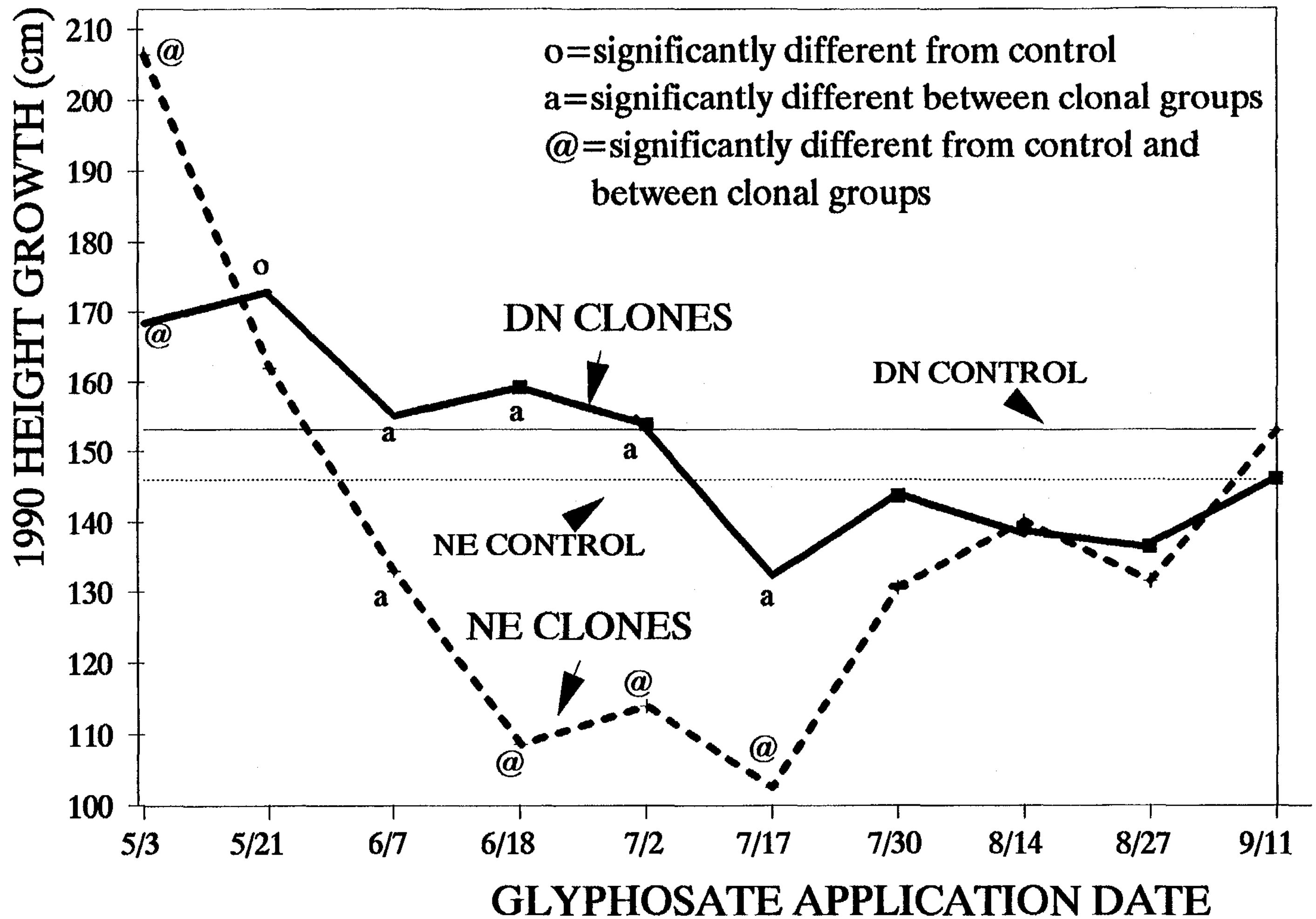
Plantation Weed Control:

Competition control is critical in establishing productive hybrid poplar plantations. Combinations of chemical and mechanical weed control strategies developed at the Harshaw Forestry Research Farm were implemented in the regional plantation network (Netzer and Hansen 1995). The preemergent herbicide linuron at one- to two-pounds active ingredient per acre is used during the first six weeks of plantation establishment. This chemical remains the only registered preemergent herbicide for hybrid poplar plantations. If linuron becomes ineffective before the trees are about 6-inches tall, a rotary hoe is operated over the trees to physically remove weeds. As the trees become taller than 6 inches small narrow discs of 5 and 6 foot in width are the most effective implement to control weeds. These discs are easy to transport and provided control of weeds in a variety of soil types. Small sprayers (less than 50 gallons) are mounted on top of these discs and used to apply grass herbicides such as sethoxydin at one-two pints per acre within the tree rows as the disc was operated between the rows.

Perennial grasses not controlled during the growing season are controlled by broadcast applications of glyphosate during late fall or spring when the hybrid poplars are dormant but the grasses are still active. This technique was developed through research conducted on four year old and younger plantations as well as coppice stool beds in 1990 (Netzer and Hansen 1992). Glyphosate at the rate of one pound active ingredient per acre was applied using commercial application techniques with no attempt to shield trees from the glyphosate spray mix. Chemical applications were made at two week intervals starting May 3 through September 11 with growth and damage recorded through the 1991 growing season. Significantly greater height growth occurred for the early May application date and significant height growth loss was noted for some clones following the June and July application dates (Figure 5). Application of glyphosate with the same rate and timing schedule, to coppice stool beds in July and August showed damage so severe to warrant this technique as a way to kill unwanted stoolbeds.

Sulfometuron (Oust) has also shown promise in dormant season applications in hybrid poplar plantations (Netzer 1995). This chemical has provided season-long weed control of most grasses and broadleaf weeds. Four trials were conducted in separate plantings in Wisconsin and Minnesota starting in 1987. A single application was made to each plantation without shielding the trees, similar to methods that might be used in commercial operations. Fall applications were made at rates from one- to three-ounces per acre (0.75 to 2.25 ounces active ingredient per acre) on October 23 when hybrid poplars were completely dormant. Spring applications at rates of one and two ounces per acre (0.75 and 1.5 ounces active ingredient per acre) were made April 18 and April 24. On the April 18 date the trees appeared completely dormant but on April 24 the trees had green leaf tips emerging from the buds. At each of the four sites height growth measurements, weed control, and tree damage observations were

Figure 5 Hybrid poplar height growth related to date of glyphosate application during the third growing season (1990). Height growth is average of four DN clones and four NE clones.



made at the end of the growing season following herbicide application. Herbicide rates and clones were replicated in some but not all trials. Spray dates were confounded by location. These trials suggested that low rates of sulfometuron, 70 grams per hectare (1 ounce product or 0.75 ounces active ingredient per acre), applied when hybrid poplar are completely dormant can provide season long control of weeds and result in increased hybrid poplar growth. Growth increases may be greater than from mechanical cultivation in drought years or dry areas. Damage to small trees and trees younger than one year may occur from broadcast applications if even slight growth activity is present. A few weeds may escape sulfometuron at low rates. Thistle seems most resistant to this herbicide and presents the greatest challenge to its use. Control of patches of thistle may be possible with directed spray of a contact herbicide like glyphosate. Late fall application of sulfometuron may be better than spring application if field access prior to spring weed growth is a concern.

Genetic and Pathogenic Variability Within Septoria musiva.

Host-parasite interactions are complex. Disease only develops if a pathogenic organism, a susceptible host, and a conducive environment are present. Screening poplars for resistance to disease requires a thorough understanding of these relationships and is further complicated by the age and size of trees, and expected rotation length. Septoria leaf spot, resulting in premature defoliation, and Septoria canker, resulting in stem breakage, are limiting diseases in many regions of North America. While our understanding of the biology of Septoria leaf spot and canker is fairly complete, our knowledge of the variability and genetics of the pathogen is extremely limited. In order to screen poplar clones for resistance we need to know the extent of pathogenic variability and distribution of pathotypes to reliably identify highly resistant trees and rank clones for their suitability as a biomass source when planted over a wide geographic region.

Collection of Septoria Isolates: A total of 859 Septoria musiva and Septoria populicola isolates have been collected. The collection consists of isolates recovered from 50 different hybrid poplar and aspen clones (diploid and triploid aspen), and native poplars (Populus alba, P. balsamifera, P. deltoides, P. trichocarpa, and P. tremuloides). Isolates were collected from 21 locations in 8 states (MN, WI, SD, ND, IA, PA, OR, and WA) and in Canada.

Growth and Morphological Characteristics on Artificial Media: Various morphometric measurements were made on a selected group of 162 isolates. Isolates were highly variable in color, sporulation, morphology, and growth rate on various media. The overall mean diameter increase after 8 days was 7.8 mm, ranging from 1.1 to 14.8 mm.

Leaf Disk Pathogenicity Assays: Leaf disks from poplars varying in susceptibility to Septoria were inoculated with 27 different isolates collected from 7 states (IA, MN, PA, OR, SD, WA, WI). On the average, green leaf tissue remaining 32 days after inoculation was 76% for the resistant clone and 46% for the susceptible clone. Isolates varied widely in pathogenicity, green leaf area remaining ranging from 0-94% on the susceptible clone and from 34-97% on the resistant clone. This is an indication that host specificity may need to be considered in this pathosystem before screening clones for resistance.

Host Differential Inoculations: Stem inoculations of poplar clones varying in susceptibility were done in the greenhouse using 10 isolates collected from 8 hybrid clones and native balsam poplar. After 14 days variation in canker development was expressed among the clones and isolates. Cankers ranged from 40-160 mm^2, with some of the smaller cankers appearing to be inactive and walled-off by the host. Some isolates were more pathogenic on the susceptible clone than on the resistant clone, however, isolates recovered from clone NE 252 and I 45/51 were highly pathogenic on both. Other isolates, particularly one recovered from clone NE 242, was more pathogenic on the resistant clone than the susceptible clone. Recommendation: ADDITIONAL RESEARCH IS NEEDED BEFORE ISOLATES CAN BE CHOSEN TO OBTAIN RELIABLE RESULTS IN RESISTANCE SCREENING TRIALS.

Molecular Marker Studies: Randomly amplified polymorphic DNA (RAPD) markers based on the polymerase chain reaction were used to detect genotypic differences among 235 isolates collected from various poplar hosts and geographic locations. Two Operon Primers have been used, F-10 (5'GGAAGCTTGG3') and F-12 (5'ACGGTACCAG3'), and both have revealed a high number of fragments. DNA fragment size has ranged from 506-4,072 bp, and the number of fragments detected per isolate has ranged from 1-12. Fragment size among isolates is variable, however, there appears to be some "family" bands which are shared by groups of isolates. These are noticeable at 1,018, 1,327, and 2,036 bp, and may prove useful in distinguishing among Septoria species.

Genetics Study: Work has been initiated on studying the heritability of markers in S. musiva. Single ascospores are being isolated from individual asci in perithecia formed in infected leaves and RAPD analyses will be done to study dominance and recessiveness of various markers.

Increase Resistance of a Selected Poplar Clone to Infection by Septoria musiva.

Tree improvement for such traits as disease resistance is a long-term, expensive activity. Tissue culture-generated variation and various rapid selection strategies have been used in many agronomic crops, and recently in forest trees to improve desired traits. Somaclonal selection for increased resistance to Septoria leaf spot has shown promise and may be a valuable strategy when used within the context of a classical breeding program for poplars.

We previously used tissue culture methods to increase the resistance of clone NE 299 (P. nigra var betulifolia X P. trichocarpa). Based on the results of a laboratory bioassay, 15% of the regenerated plants from callus cultures had increased resistance to S. musiva. Because of its rapid early growth and apparent tolerance to drought conditions, clone NE 308 (P. charkowiensis X P. incrassata) was chosen to further test somaclonal selection as a way to rapidly improve its resistance to Septoria leaf spot and canker. NE 308 is quite susceptible to Septoria leaf spot and canker in the field and therefore cannot be recommended for planting at this time. If somaclonal variation in disease resistance can be utilized in this clone as it was in NE 299, then the somaclonal selection strategy would be worth pursuing further with additional clones.

The tissue culture and plant regeneration systems needed to handle this genotype were optimized. Clone NE 308 proved to be much more difficult to propagate in vitro than many of the other poplar clones tested. Plantlet regeneration, rooting, and acclimation of NE 308 were difficult as well, supporting our previous results indicating extreme variability in success of tissue culture within Populus clones and species.

During the course of this study over 900 adventitious shoots from callus were generated from NE 308. Of these, over 500 plants (somaclones) survived rooting and acclimation to greenhouse conditions. Many mutant plants died soon after being moved to greenhouse conditions, and others died from various other causes. In addition, 356 plants regenerated from axillary buds, and plants regenerated from hardwood and softwood cuttings of NE 308 were established in the greenhouse. Plants regenerated from axillary buds and cuttings served as control plants in the bioassays and will be outplanted with selected somaclones for field testing. In previous work it was found that axillary bud culture could induce somatic variation in Populus, indicating that the tissue culture cycle itself may result in instability of some clones when grown in vitro.

All NE 308 somaclones have been tested at least twice for resistance to Septoria leaf spot using the leaf disk laboratory bioassay. Thus far, with only a few plants needing re-testing, out of 486 somaclones we have recovered 29 plants that have tested resistant twice. The resistant somaclones averaged over 20% greater green leaf area 32 days after inoculation. In addition, disease progression on leaf disks of several somaclones was delayed compared to the donor clone which may serve to delay sporulation and thus limit disease spread. Most regenerated plants, however, tested as susceptible as the donor (non tissue-cultured) plant. We have propagated several of these which also will be included in our field test for comparison of their disease reactions and growth characteristics to the resistant somaclones and the donor clone.

To be considered true somaclonal variants, genetic analyses must be done. At Rhinelander, WI we have NE 299 somaclones that have exhibited resistance to Septoria canker since they were planted in 1986. All plants of NE 299 in this planting that did not undergo a tissue culture cycle have been killed by Septoria canker. These trees are being maintained in the test plot and will be allowed to flower. Although breeding work has not been done with somaclones with putative resistance to Septoria leaf spot and canker, tests are underway to determine if the disease resistance trait is stable after vegetative propagation. Plants vegetatively propagated using conventional cuttage techniques have been under test at Rosemount, MN. Also, we have over 200 "generation 2" NE 308 plants. These are plants propagated using softwood cuttings of selected somaclone lines. Field testing of this plant population will begin in the summer in a replicated planting at Rosemount, MN.

Somaclones of NE 299 have been field-tested under natural inoculum levels and cultural conditions. Trees have been assessed for growth, form, and most importantly, resistance to all major insects and pathogens in addition to S. musiva. Somaclones of NE 308 will undergo similar testing. Selected trees will then be propagated and will be made available to collaborators for further testing and potential breeding.

Related Research Supporting Short Rotation Woody Crop Trials

Poplar Clonal Trials: Disease Screening: Poplar selections in the clonal trials established in the region have been monitored for resistance to the major limiting diseases. This work has resulted in the identification of a group of clones that have exhibited good growth and a high level of resistance or tolerance to disease over a range of sites. Of importance is the detection of some site differences in relation to disease incidence and disease severity. Available soil moisture may play a critical role in disease expression and impact. The common set of clones across a wide range of sites also allows monitoring of the pathogen populations affecting trees. Collections of various fungi are being made from selected clones in several of the trials for identification and comparison purposes.

Bird and Small Mammal Use of Hybrid Poplar Plantations: A separately funded study is investigating the effect of replacing traditional agricultural row, grain and hay crops with perennial tree plantations. The question is one of determining the relative value of tree crops for food, cover and nesting for a variety of birds and mammals as compared to the nominal agricultural landscape.

Biological Control of Septoria Leaf Spot and Canker: Laboratory assays using the bacterium Streptomyces scabies demonstrated antibiotic activity against many of the S. musiva isolates collected during the course of our research. Variation in antibiotic activity among S. scabies isolates was evident as was variability in the level of inhibition of isolates of S. musiva by a given S. scabies isolate. S. scabies is found in soil and on infected plant debris, and may be an effective control agent on many sites where poplars are being grown. Reduction of over-wintering inoculum on infected leaves will most likely reduce disease incidence and severity the following spring. It may especially be effective as a preventative treatment in nursery stool beds and on planting stock.

Detection and Identification of Poplar Leaf Rusts: In conjunction with the International Energy Agency Bioenergy Agreement Task Working Groups on Pest/Disease Control and Exchange, a common set of poplar clones have been planted in Wisconsin, Minnesota, Iowa, and Washington with the objective of detecting and monitoring the leaf rust populations present over this geographic range. We now know that several species and races of Melampsora are present in the poplar-growing regions of North America and that serious disease outbreaks caused by newly-evolved or introduced pathotypes can occur. The goal is to gain knowledge of the distribution of the various rust populations so that effective rust resistance screening can be undertaken.

CONCLUSIONS

1. Mean annual biomass production for the five best clones over the eight test sites averages 3 dry tons per acre per year (TAY) .
2. The best clone (DN-34) averages 3.3 TAY over all sites.
3. The best site (Granite Falls) averages 3.9 TAY over all clones.
4. The best clone at the best site yields 4.6-4.7 TAY.
5. Other clones in the small clonal trial plots have yields up to 8.1 TAY.
6. Biomass yields are still increasing at most sites through year 8.
7. Biomass yields would probably be somewhat higher if the high mortality during the establishment year had been avoided.
8. Establishment year mortality was 27 percent. Subsequent mortality of the best four clones was only 2 percent through year 8.
9. Reduced growth at some sites was related primarily to insufficient soil water and susceptibility to the disease Septoria musiva.
10. Fertilization at 75 and 150 pounds per acre N resulted in significant increases in leaf tissue N.
11. Leaf N remained elevated through the first growing season following fertilization, but early results indicate that effects probably do not carry over to the second year.
12. No significant increase in tree growth following fertilization has been detected.
13. Clones with the best survival and growth are predominantly of deltoides-nigra parentage; other clones that are growing well are either nigra-maximowicsii, or pure deltoides parentage.
14. The same clones are the best growers on both "good" and "harsh" sites.
15. Most clones from the northeast U.S. (NE) are severely diseased and some have died completely at some sites.
16. A collection of 859 Septoria spp. isolates has shown considerable variability in the microorganism.
17. Inoculation tests indicate that host specificity may need to be considered when screening clones for resistance.

18. Hybrid poplar plantations grown on tilled agricultural lands previously in prairie, sequester significant quantities of soil carbon.

19. Establishment and tending of the plantation can lead to early soil carbon loss, but this is replaced as the plantation ages.

20. Glyphosate can be applied safely to poplar plantations during the tree dormancy periods in early spring and late fall.

21. Sulfometuron has shown promise for season-long weed control from a single application during the tree dormancy periods in early spring and late fall.

22. About half of the total cost for tending the plantations through year 8 ($470-$660) was required for the first year establishment and tending cost.

RECOMMENDATIONS

1. Continue annual biomass measurements until biomass production has peaked at most sites.

2. Measure leaf N on all plot in the Milaca fertilization test in 1995 and analyze for duration of fertilizer response.

3. Measure tree diameter on all plots in the Milaca fertilizer test in 1995 and analyze for growth response to N fertilization.

4. Continue annual growth, survival and disease assessment of the hybrid trials until trials with new genetic material from ISU breeding program have material available for commercial release.

5. Additional research is needed before isolates can be chosen to obtain reliable results in resistance screening trials.

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LOCATION: ASHLAND, WI - 1987 (1-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Site Preparation	Mechanical	Moldboard plowing	11/86	0	60 HP tractor w/3 bottom moldboard plow	Farmer (contract)	$30/hr	$135.00	$13.50
B	10	10	Site Preparation	Mechanical	Discing	4/87	1	60 HP tractor with 10 foot wide disc	Farmer (contract)	$30/hr	$105.00	$10.50
C	10	10	Site Preparation	Chemical	Linuron tretment	4/87	1	26 HP tractor with 30 foot boom sprayer	Farmer (contract)	$10/acre	$100.00	$10.00
					Linuron chemical 2 lbs/acre				Coop	$6.10/lb	$122.00	$12.20
D	10	10	Planting	Planting	Hand planting	4/87	1	Hand planting at 8'x8'	Contract crew	$0.0975/ cutting	$656.37	$66.60
			Cuttings						5.5 hours 5 persons	$0.09/ cutting	$605.88	$61.20
E	10	10	Tending	Mechanical	Cultivation in two directions	7/87	1	26 HP tractor w/7' disc	NCFES 9.5 hrs	$18.00	$171.00	$17.10
F	10	10	Tending	Mechanical	Cultivation in two directions	8/87	1	26 HP tractor w/7' disc	NCFES	$18.00	$180.00	$18.00
G	10	10	Tending	Chemical	Linuron/glyphosate	11/87	1	26 HP tractor w/42' boom	NCFES	$20.00/hr	$80.00	$8.00
					Linuron chemical			2 pints/acre		$74.00/ga	$185.00	$18.50
					Glyphosate chemical			3 pints/acre		$59.54/ga	$219.71	$21.97

LOCATION: SIOUX FALLS, SD - 1987 (1-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Site preparation	Mechanical	Chisel plow followed by 2 passes with field cultivator	4/87	1	75 HP tractor w/10' chisel plow	Farmer (contract)		$100.00	$10.00
B	10	10	Site Preparation	Chemical	Linuron treatment	4/87	1	75 HP tractor w/30' boom sprayer	Farmer (contract)		$40.00	$4.00
				Linuron	Linuron chemical			Linuron chemical	Coop		$275.00	$27.50
C	10	10	Planting	Planting	Hand planting	4/87	1	Hand planting at 8'x8'	Contract crew (5)	9.75 cents/ cutting	$663.00	$66.30
			Cuttings					680 trees/acre Cuttings		9 cents ea	$612.00	$61.20
D	10	10	Tending	Chemical	Fluazifop treatment	6/87	1	75 HP tractor w/30' boom sprayer	Farmer (contract)		$40.00	$4.00
					Fluazifop chemical			Fluazifop chemical	Coop		$174.00	$17.40
E	10	10	Tending	Chemical	Glyphosate spot spray	6/87	1	1 person @ $4.50/hr	Farmer (contract) Coop		$101.25	$10.13
					Glyphosate chemical			Glyphosate chemical			$62.50	$6.25
F	10	10	Tending	Mechanical	Mowing between tree rows	7/87	1	18 HP tractor w/5 foot wide rotary mower	Farmer (contract)		$50.00	$10.00
G	10	10	Tending	Mechanical	Mowing between tree rows	8/87	1	18 HP tractor w/5 foot wide rotary mower	Farmer (contract)		$20.00	$4.00
H	10	10	Tending	Hand Weeding	Hand weeding	7,8/87	1	4 persons @ $5.00/hr.	Farmer		$577.50	$57.50
I	10	10	Tending	Chemical	Cottonwood leaf beatle control	8/87	1	Backpack mist blower	NCFES		$17.00	$1.70
								Malathion chemical			$10.16	$1.02
J	10	10	Tending	Chemical	Linuron treatment	11/87	1	26 HP tractor w/42' boom	NCFES		$70.00	$7.00
					Linuron chemical			2 pints/acre		$74.00/ga	$185.00	$18.50

ASHLAND, WI - 1988 (2-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Tending	Mechanical Chemical	Disc between rows Spray fusilade w/in rows	6/88	2	40 HP tractor with 6 foot wide disc/ sprayer	NCFES	$4.10/ac	$41.40	$4.10
	10	10	Tending	Chemical	Fusilade chemical (16 oz/acre)			Fusilade chemical	Coop	$73.16/ga	$91.45	$9.15
B	10	10	Tending	Mechanical	Disk between rows in two directions	7/9/88	2	40 HP tractor with 5 foot wide disc	NCFES	$4.10/ac	$82.00	$8.20

LOCATION: SIOUX FALLS, SD - 1988 (2-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Tending	Mechanical	Disc between rows two directions	6/88	2	40 horsepower tractor with 6' disc	NCFES	$8/ac	$80	$8.00
B	20	20	Tending	Chemical	Aerial malathion application	7/88	2	Fixed wing aircraft (cropduster)	Farmer (contract)	$7.54	$150.75	$7.54
C	10	10	Tending	Mechanical	Mow weeds in rows	6/88	2	50 HP tractor with 6' rotary mower	NCFES	$6.43	$64.30	$6.43
D	10	10	Tending	Mechanical	Mow weeds in rows	9/88	2	50 HP tractor with 6' rotary mower	NCFES	$6.43	$64.30	$6.43

LOCATION: ASHLAND, WI - 1989 (3-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Tending	Chemical	Spring glyphosate overspray	5/89	3	50 HP tractor with 42' spray boom	NCFES	$4.00/ac	$40.00	$4.00
B	10	10	Tending	Chemical	Roundup chemical	5/89	3	1 lb/acre (1 qt/acre)	Chemical company	$54.59/ga	$136.48	$13.48
C	10	10	Tending	Mechanical	Disk between rows in one direction	6/89	3	50 HP tractor with 6' disk	NCFES	$4.10/ac	$41.00	$4.10
D	10	10	Tending	Mechanical	Disk between rows	7/89	3	50 HP tractor with 6' disk and attached sprayer	NCFES	$4.10/ac	$41.00	$4.10
				Chemical	Spray Fusilade within rows							
E	10	10	Tending	Chemical	Fusilade chemical	7/89	3		Chemical company	$73.16/ga	$91.45	$9.15
F	10	10	Tending	Mechanical	Mow between rows in one direction	8/89	3	50 HP tractor with 6' rotary mower	NCFES	$6.43/ac	$64.30	$6.43
G	10	20	Tending	Mechanical	Disk between rows in 2 directions	8/89	3	50 HP tractor with 6' disk	NCFES	$4.10/ac	$41.00	$4.10

LOCATION: SIOUX FALLS, SD - 1989 (3-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Tending	Mechanical	Disk between rows in one direction	6/89	3	50 HP tractor with 5' disk	NCFES	$4.10/ac	$41.00	$4.10
B	10	10	Tending	Mechanical	Mow between rows in one direction	7/89	3	50 HP tractor with 6' rotary mower	NCFES	$6.43/ac	$64.30	$6.43
C	10	10	Tending	Mechanical	Mow between rows in two directions	9/89	3	50 HP tractor with 6' disk	NCFES	$4.10/ac	$82.00	$8.20
D	10	10	Tending	Chemical	Apply granular simazine	11/89	3	50 HP tractor with attached cyclone type spreader	NCFES	$5.00/ac	$50.00	$5.00
E	10	10	Tending	Chemical	Simazine chemical	11/89	3	Simazine applied at 100 lbs/acre	Chemical company	$1.21/lb	$1210.00	$121.00

LOCATION: ASHLAND, WI - 1990 (4-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	10	Tending	Chemical	Granular herbicide application	4/90	4	4-wheeler with attached cyclone-type spreader	NCFES	$5.48/ac	$54.80	$5.48
B	10	10	Tending	Chemical	Simazine granular	4/90	4	3 lb/acre active ingredients 75 lbs/acre bag mix	Chemical Company	$1.40/lb	$1050.00	$105.00

LOCATION: SIOUX FALLS, SD - 1990 (4-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	9	9	Tending	Chemical	Directed glyphosate spray	6/90	4	4-wheeler with 6 foot wide boom trailer sprayer	NCFES	$5.48/ac	$49.32	$5.48
B	9	9	Tending	Chemical	Glyphosate (Roundup)	6/90	4		Chemical company	$54.59/ga	$122.83	$13.64
C	9	4	Tending	Mechanical	Cut thistles with hand-held weed mower	6/90	4	Motorized hand-held weed cutter	NCFES	$15.00/ac	$60.00	$15.00

1991 TENDING COSTS FOR TWO 5-YEAR-OLD HYBRID POPLAR PLANTATIONS

LOCATION: ASHLAND, WISCONSIN - 1991 (5-YEAR-OLD PLANTATION)

OPERATION	TOTAL ACRES	TREATED ACRES	ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YR	MACHINERY USED	PROVIDER	UNIT COST	COST PER TOTAL COST	PROJECT ACRE
A	10	4	Tending	Mechanical	Mow open areas in and around planting	7/91	5	28 HP tractor with 6 ft. rotary mower	NCFES	$15.00/ac	$150.00	$ 15.00

LOCATION: SIOUX FALLS, SOUTH DAKOTA - 1991 (5-YEAR-OLD PLANTATION)

OPERATION	TOTAL ACRES	TREATED ACRES	ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YR	MACHINERY USED	PROVIDER	UNIT COST	COST PER TOTAL COST	PROJECT ACRE
A	9	9	Tending	Chemical	Spring glyphosate application	5/91	5	4-wheeler with 6 ft-wide spray boom sprayer	NCFES	$5.48	$49.32	$ 5.48
B	9	9	Tending	Chemical	Glyphosate	5/91	5	1 lb/ac (1 qt/ac)	Chemical Company	$54.59/ga	$122.83	$ 13.64
C	10	10	Tending	Chemical	Cottonwood leaf beetle	6/91	5	Fixed-wing aircraft	Contract	$10.00/ac.	$100.00	$ 10.00
D	10	10	Tending	Chemical	Malathion ULV	6/91	5	1 qt/acre Company	Chemical	$26.45/ga	$66.13	$ 6.61
E	9	9	Tending	Mechanical	Mow thistle between rows	7/91	5	28 HP tractor with 6 ft rotary mower	NCFES	$15.00/ac	$135.00	$ 15.00
F	9	9	Tending	Mechanical	Disk in two directions	9/91	5	28 HP tractor with 6 ft-wide disk	NCFES	$15.00/ac	$270.00	$ 30.00

LOCATION: ASHLAND, WI - 1992 (6-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	5	Tending	Chemical	Spring glyphosate application	5/92	6	28 HP tractor 6 foot wide boom sprayer	NCFES	$5.48/ac	$27.40	$5.48
B	10	5	Tending	Chemical	Roundup chemical	5/92	6	1 lb/acre (1 qt/acre)	Chemical Company	$54.59/ga	$68.23	$13.64

LOCATION: SIOUX FALLS, SD - 1992 (6-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	9	9	Tending	Chemical	Spring glyphosate application	5/92	6	28 HP tractor with 6 foot boom sprayer	NCFES	$5.48	$49.32	$5.48
B	9	9	Tending	Chemical	Roundup chemical	5/92	6	1 lb/acre (1 qt/acre)	Chemical Company	$54.59/ga	$122.83	$13.64
C	9	9	Tending	Mechanical	Mow weeds in rows	7/92	6	28 HP tractor with 6 foot wide rotary mower	NCFES	$15.00/ac	$135.00	$15.00
D	9	9	Tending	Mechanical	Disc between rows two directions	8/92	6	28 HP ractor with 6 foot tandem disc	Contractor	$20/hr	$140.00	$15.50

LOCATION: ASHLAND, WI - 1993 (7-YEAR-OLD PLANTATION)

There were no tending activities on the plantation in 1993. A strrip around the outside edge of the planting was mowed once and the cost is noted below:

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	10	0.6	Tending	Mechanical	Mow outside edge of plantation	7/93	7	28 HP tractor 6 foot wide rotary mower	NCFES	$15.00/ac	$9.00	$15.00

LOCATION: SIOUX FALLS, SD - 1993 (7-YEAR-OLD PLANTATION)

OPERATION	ACRES	TREATED ACRES	MAJOR ACTIVITY CATEGORY	SUBACTIVITY	SPECIFIC ACTIVITY	DATE	YEAR	MACHINERY USED	PROVIDER	UNIT COST	TOTAL COST	COST PER PROJECT ACRE
A	9	9	Tending	Mechanical	Mow thistle between tree rows	8/93	7	28 HP tractor 6 foot wide rotary mower	NCFES	$15.00/ac	$135.00	$15.00

LOCATION: ASHLAND, WI - 1994 (8-YEAR-OLD PLANTATION)

There were no tending activities on the plantation in 1994.

LOCATION: SIOUX FALLS, SD - 1994 (8-YEAR-OLD PLANTATION)

There were no tending activities on the plantation in 1994.

TREE DRY WEIGHT DATA
(8X8 feet spacing)

ASHLAND (1987 plantation)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
DN 34	13.8	10.25	15.49	25.21	40.70	7
	11.5	9.77	8.86	18.76	27.62	7
	10.3	9.30	6.56	15.31	21.88	7
	9.5	9.12	4.19	11.26	15.46	7
	8.3	7.50	6.62	8.72	15.34	5
	3.1	3.55			3.51	3
	2.7	3.35			2.09	3
	2.6	3.32			2.19	3
	1.8	2.55			1.16	3
	1.2	2.23			.47	3
NE 308	10.1	9.2	5.21	12.45	17.66	5
	5.3	5.09			5.173	3
	4.2	4.36			4.314	3
	3.5	3.93			2.844	3
	2.8	3.30			2.477	3
	2.0	2.92			.968	3
DN 182	8.9	8.0	8.44	10.94	19.38	5
	3.5	4.30			4.786	3
	3.3	4.22			4.286	3
	2.2	3.21			2.173	3
	2.0	2.68			1.489	3
	1.6	2.24			.991	3
DN 17	13.7	10.96	15.16	27.64	42.80	7
	11.7	11.25	7.28	22.56	29.84	7
	10.6	10.74	4.99	17.37	22.37	7
	9.4	10.10	4.23	14.26	18.49	7
	7.8	8.10	5.48	9.25	14.73	5
	5.0	6.30	2.42	3.70	6.12	5
	4.2	4.06			3.957	3
	3.6	4.20			3.715	3
	3.2	3.88			2.850	3
	2.5	3.40			2.069	3
	2.2	3.08			1.674	3
	1.9	2.83			.716	3
SIOUX	7.5	6.7	3.75	5.85	9.60	5

GRANITE FALLS (1987 plantation)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
DN 34	19.3	15.65	22.63	70.53	93.16	8
	18.5	16.34	17.06	66.44	83.50	8
	17.5	15.25	15.78	56.61	72.40	8
	16.0	15.05	12.43	48.97	61.39	8
	2.0	3.12			.900	3
	3.2	4.56			2.007	3
	4.3	5.10			2.818	3
	5.2	5.65			4.232	3
	6.4	6.35			7.030	3
NE 308	3.2	4.61			1.338	3
	4.1	5.76			2.356	3
	5.0	6.33			3.900	3
	6.8	8.17			7.685	3
	7.6	7.41			9.699	3
DN 182	3.2	3.86			1.886	3
	4.1	5.48			3.377	3
	5.2	5.10			3.965	3
	6.7	6.89			6.936	3
	7.5	7.08			8.934	3
DN 17	2.2	3.46			1.015	3
	3.9	4.47			2.616	3
	4.7	5.62			3.216	3
	5.0	5.81			4.722	3
	6.3	6.43			7.320	3

FAIRMONT (1986 plantations)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
SIOUX	10.2	10.23			25.133	3
(3x3 m)	9.1	10.67			18.325	3
	7.2	9.60			12.209	3
	5.5	7.76			6.558	3
	15.2	12.71	12.38	37.56	49.935	6

MILACA (1987 plantations)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
NE387	11.4	8.5	10.89	14.30	25.19	5
	10.6	8.0	10.30	12.36	22.66	4
	9.1	7.8	6.01	9.56	15.57	4
	7.4	7.2	3.59	6.46	10.05	4
	6.6	6.8	2.95	4.57	7.51	4
	5.3	5.6	2.49	3.65	6.15	4
NE54	10.3	8.6	8.28	12.15	20.43	5
	6.2	7.3	3.03	4.53	7.56	5
	10.5	8.8	9.69	13.72	23.40	4
	9.5	8.6	9.98	13.12	23.11	4
	8.4	8.0	7.57	9.62	17.20	4
	6.5	7.5	3.24	4.78	8.02	4
	5.7	7.3	2.58	4.25	6.84	4
DN182	18.6	15.5	19.62	64.16	83.77	8
	16.5	15.8	12.29	48.45	60.74	8
	12.2	11.1	8.28	20.30	28.58	5
	12.0	10.0	8.82	16.79	25.61	4
	10.0	9.4	6.88	13.49	20.37	4
	5.8	7.6	1.74	4.35	6.09	4
	4.5	5.8	2.06	2.71	4.77	4
NE308	12.9	12.0	5.86	22.63	28.49	5
	12.1	9.4	7.34	16.21	23.55	4
	10.2	9.0	4.41	10.72	15.13	4
	7.8	8.0	1.89	7.71	9.60	4
	5.1	6.5	.63	2.84	3.45	4
DN17	19.95	15.97	18.81	65.52	84.33	8
	14.90	15.35	10.74	44.58	55.32	8
	13.5	11.1	9.10	24.50	33.60	5
	11.1	8.9	7.52	13.77	21.28	4
	8.8	8.5	5.84	10.27	16.11	4
	5.6	7.4	1.68	4.13	5.81	4
	3.6	5.0	.75	1.50	2.25	4
DN34	13.4	9.7	13.02	22.21	35.23	5
	9.5	7.6	6.26	9.62	15.89	4
	7.2	7.4	4.10	6.87	10.97	4
	6.1	7.2	2.05	4.36	6.41	4
	3.1	5.0	.50	1.20	1.70	4

MILACA Con't (1987 plantations)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
SIOUX	13.4	11.4	7.72	21.35	29.07	5
DN 1	8.1	8.4	4.46	8.21	12.67	5
DN 2	16.9	12.3	17.51	37.62	55.13	5
DN 5	13.3	10.9	7.16	20.94	28.10	5
DN131	9.1	10.2	3.34	11.00	14.34	5
NE222	9.8	9.6	2.99	10.61	13.60	5
I45-51	12.0	9.7	5.32	16.30	21.62	5
NM 2	13.0	11.5	10.47	23.01	33.48	5

SIOUX FALLS (1987 PLANTATION)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
SIOUX	7.0	5.4	4.20	5.67	9.87	5
NE308	9.0	7.2	4.10	10.93	15.03	5
DN182	9.0	7.4	5.32	10.96	16.28	5
DN34	12.2	10.31	6.93	20.42	27.35	7
DN34	11.0	10.13	4.81	16.34	21.05	7
DN34	9.9	7.4	8.02	13.31	21.33	5
DN17	13.6	11.05	12.10	26.94	39.04	7
DN17	11.5	10.55	6.62	17.43	24.05	7
DN17	9.1	7.5	6.05	10.74	16.80	5

FARGO (1987 PLANTATION)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE yr
DN182	14.6	11.12	15.25	31.40	46.65	8
	15.2	10.85	11.04	33.63	44.68	8
DN17	16.7	11.73	12.85	36.58	49.42	8
	17.7	12.15	12.50	35.80	48.30	8

MONDOVI (1987 plantation)

CLONE	DBH cm	HGT m	BRANCH kg	STEM kg	TOTAL kg	AGE
DN182	12.7	13.1	8.46	25.65	34.13	5
	11.1	10.3	8.56	17.06	25.61	4
	9.4	9.9	4.02	11.25	15.27	4
	5.7	7.3	1.44	3.79	5.23	4
	3.8	5.6	.71	1.81	2.52	4
NE308	12.0	12.4	9.51	27.23	36.74	5
	10.2	10.2	7.38	15.92	23.30	4
	8.6	10.6	2.32	9.48	11.79	4
	6.8	8.9	1.63	6.19	7.82	4
	4.6	7.0	.56	2.48	3.04	4
DN17	17.8	17.08	16.12	62.09	78.22	7
	16.3	16.73	12.15	51.89	64.04	7
	15.5	15.35	12.98	46.61	59.58	7
	14.3	16.15	9.00	38.87	47.87	7
	13.1	12.80	11.60	30.03	41.63	4
	10.2	9.8	6.20	14.43	20.62	4
	8.8	9.0	3.89	9.02	12.91	4
	5.2	8.0	.96	3.64	4.60	4
	3.7	5.7	.50	1.51	2.02	4
DN34	16.8	14.50	16.20	52.36	68.56	7
	15.7	15.31	11.10	49.98	56.08	7
	14.6	15.74	11.40	44.56	55.96	7
	11.2	11.3	8.38	20.27	28.65	5
	9.2	9.4	5.89	12.19	18.08	4
	8.1	8.8	3.42	8.60	12.02	4
	6.1	7.4	1.63	4.47	6.10	4
	4.3	5.5	1.19	2.40	3.59	4
SIOUX	12.3	11.9	10.20	22.86	33.01	5

Number of Septoria Isolates Recovered From Each Host Species

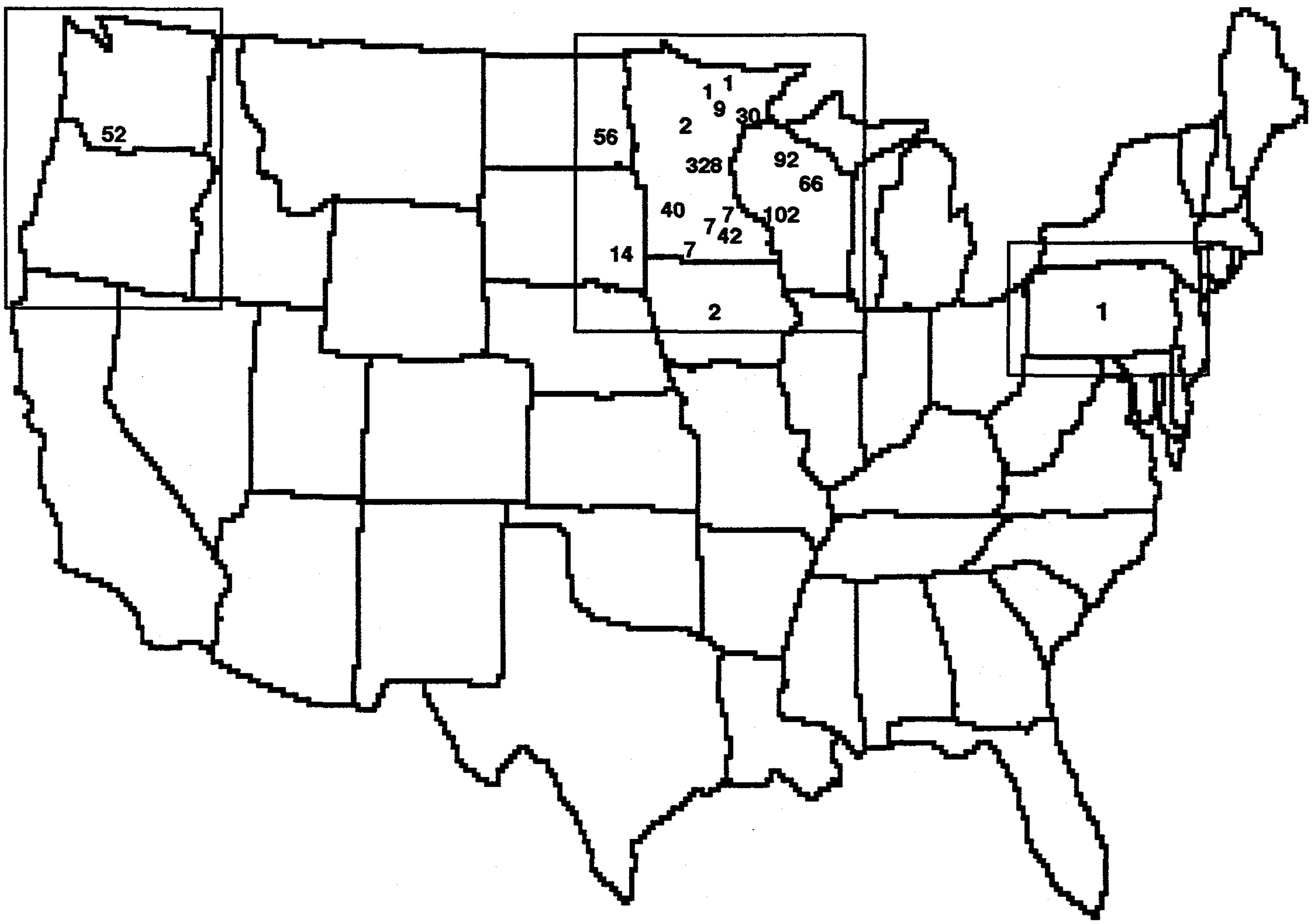
Host	Quantity	Host	Quantity
Aspen	20	DTAC 16	20
Diploid aspen	1	DTAC 26	24
Triploid aspen	6	I 45-1	4
Balsam poplar	44	I 45/51	26
Cottonwood	35	JACKII 4	37
P. alba	7	NC 5260	19
P. trichocarpa	59	NE 17	4
Somaclone	6	NE 19	19
Hybrid poplar	9	NE 27	5
DN 16	16	NE 28	17
DN 17	2	NE 41	3
DN 19	1	NE 47	3
DN 30	1	NE 50	4
DN 34	23	NE 51	3
DN 55	4	NE 54	19
DN 65	1	NE 56	27
DN 70	26	NE 242	42
DN 74	8	NE 252	20
DN 93	2	NE 258	5
DN 131	3	NE 299	19
DN 160	1	NE 300	1
DN 174	1	NE 308	38
DN 182	2	NE 351	27
DTAC 4	1	NM 2	101
DTAC 7	17	NM 6	54
DTAC 8	1	SIOUXLAND	11
DTAC 9	3	47-174	1
DTAC 10	6		
		Total	859

Number of Septoria Isolates Collected at Each Site

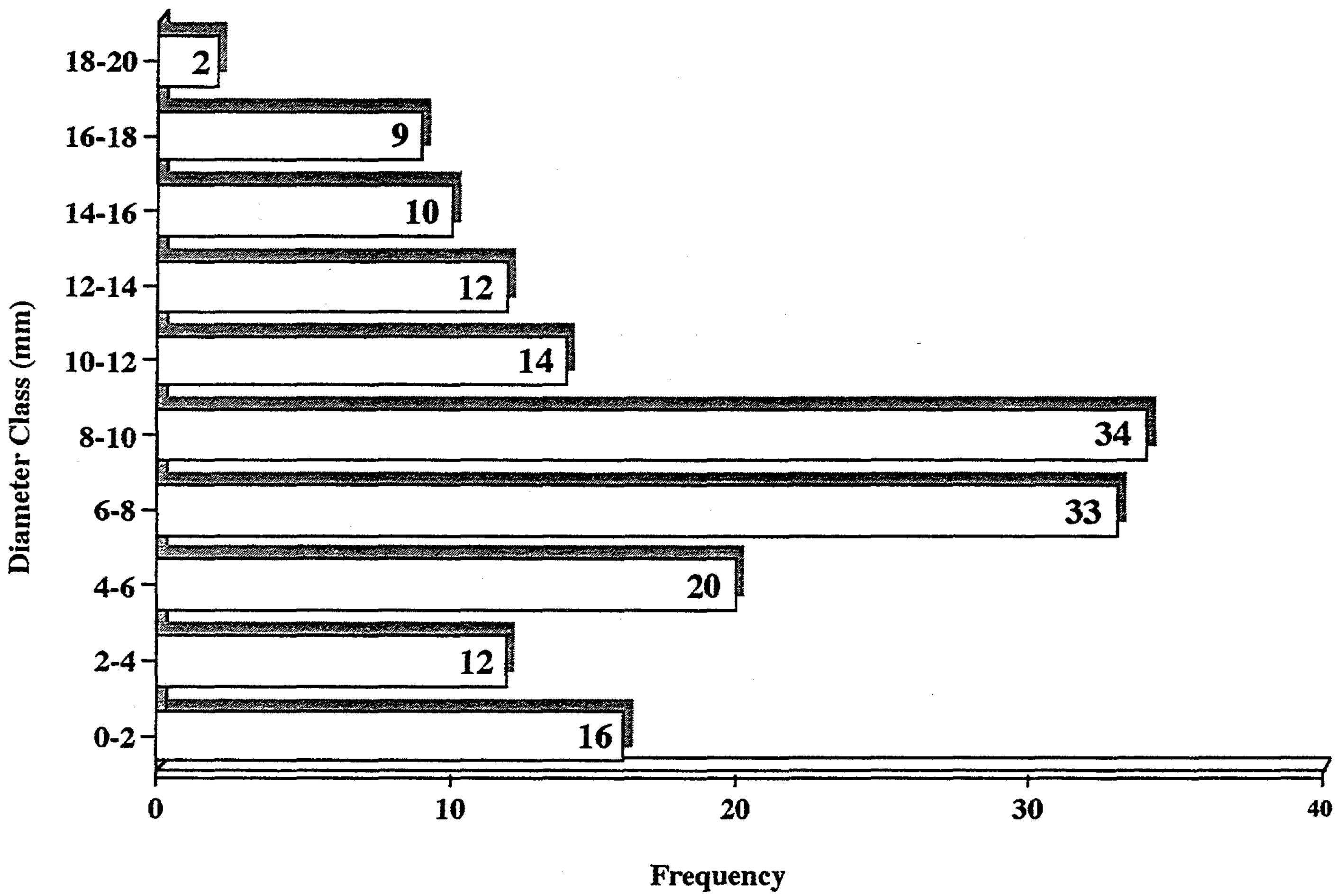
Location	Quantity	Location	Quantity
Canada		**North Dakota**	
Ontario	1	Fargo	56
Iowa		**South Dakota**	
Ames	2	Sioux Falls	14
Minnesota		**West Coast**	
Chippewa NF	2	Oregon / Washington	52
Cloquet	30		
Fairmont	7	**Wisconsin**	
Grand Rapids	9	Ashland	92
Granite Falls	40	Mondovi	102
International Falls	1	Rhinelander	66
Milaca	328		
Northome	1		
Rosemount	42		
Savage	7		
St. Paul	7		
		Total	859

Geographic Distribution of Septoria spp. Isolate Collection

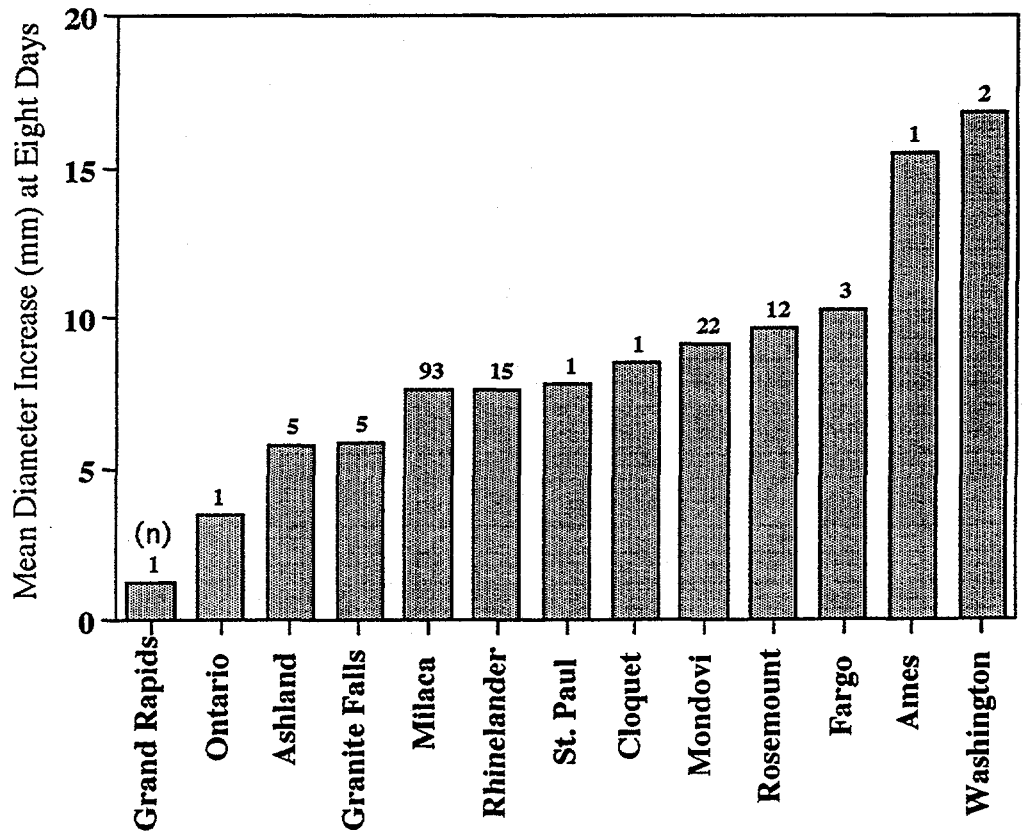
Number of Isolates Recovered at Each Location



Growth by Diameter Class of 162 Septoria Isolates 13 Days After Inoculation



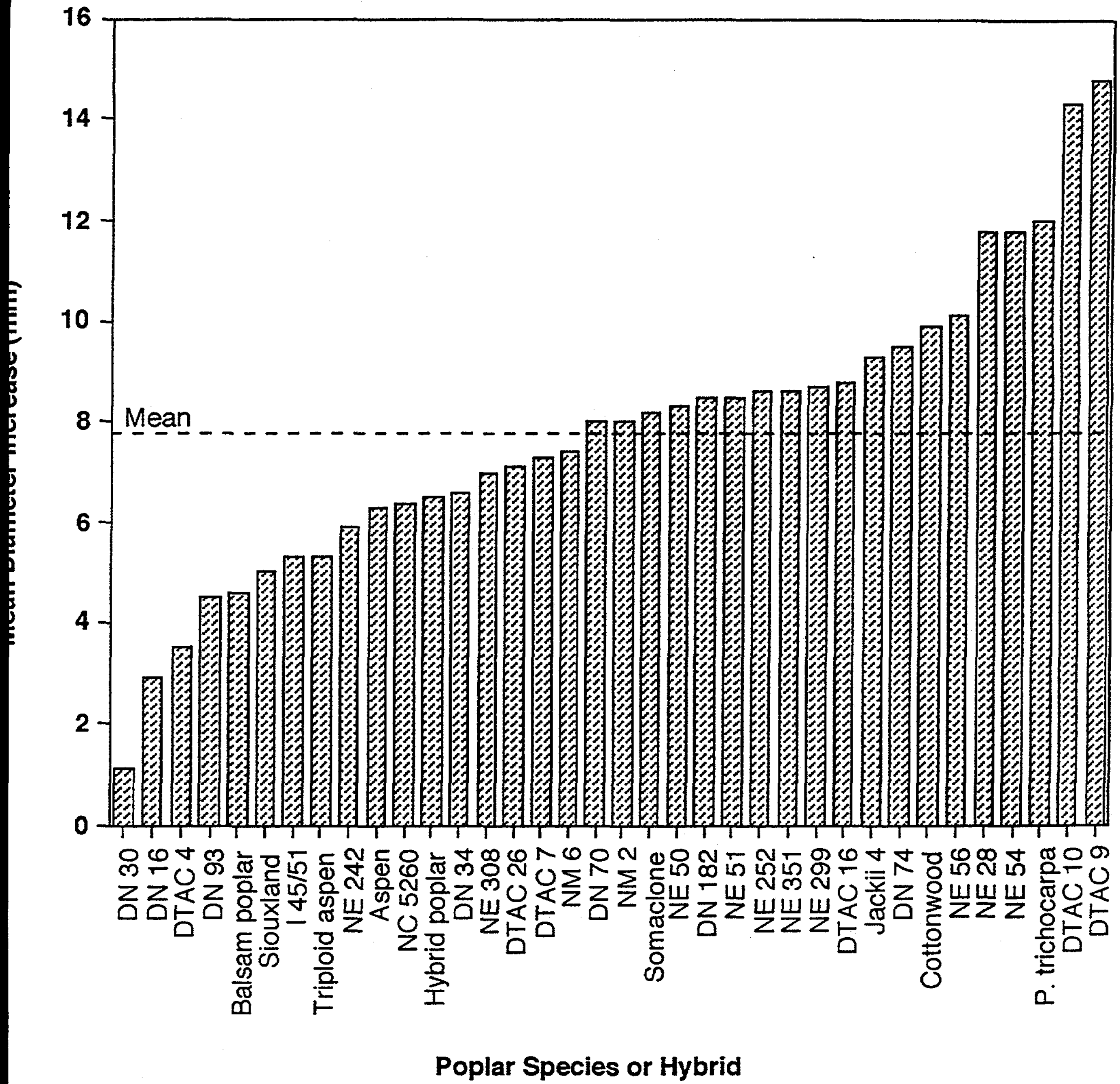
Growth of 162 Septoria spp. Isolates Collected From Different Sites



Growth of 162 Septoria spp. Isolates Collected From Various Populus Species and Hybrids

Populus Species or Hybrid	Number of Isolates	Mean Diameter Increase (mm) after 8 Days
DN 30	1	1.1
DN 16	4	2.9
DTAC 4	1	3.5
DN 93	1	4.5
Balsam poplar	2	4.6
Siouxland	1	5.0
I45/51	6	5.3
Triploid aspen	1	5.3
NE 242	8	5.9
Aspen	1	6.3
NC 5260	2	6.4
Hybrid poplar	5	6.5
DN 34	2	6.6
NE 308	6	7.0
DTAC 26	3	7.1
DTAC 7	4	7.3
NM 6	14	7.4
DN 70	11	8.0
NM 2	22	8.0
Somaclone	2	8.2
NE 50	1	8.3
DN 182	1	8.5
NE 51	1	8.5
NE 351	9	8.6
NE 252	4	8.6
NE 299	4	8.7
DTAC 16	3	8.8
Jackii 4	16	9.3
DN 74	1	9.5
Cottonwood	3	9.9
NE 56	8	10.1
NE 28	3	11.8
NE 54	3	11.8
P. trichocarpa	2	12.0
DTAC 10	5	14.3
DTAC 9	1	14.8
	Mean	7.8

Growth of 162 Septoria spp. Isolates Collected From Different Poplars



Comparison of Pathogenicity of Different Septoria Isolates using a Leaf Disk Assay

Septoria Isolate	Collection Location	DN 34 (resistant) Leaf Reaction (% green at 32 days)	NE 299 (susceptible) Leaf Reaction (% green at 32 days)
648	Pennsylvania	34	24
523	Minnesota	44	19
598	Wisconsin	46	33
534	Minnesota	49	75
567	South Dakota	55	37
599	Wisconsin	66	87
514	Minnesota	67	32
619	Oregon	70	6
486	Minnesota	73	11
611	Minnesota	75	25
SM3	Iowa	76	56
584	Minnesota	76	54
606	Wisconsin	77	0
652	Washington	80	94
403	Minnesota	83	4
589	Minnesota	85	64
650	Wisconsin	87	8
612	South Dakota	88	78
513	Minnesota	88	41
379	Minnesota	88	42
592	Minnesota	89	41
653	Washington	92	40
605	Wisconsin	92	94
554	Minnesota	94	77
488	Minnesota	95	93
485	Minnesota	96	57
SM2	Iowa	97	42
	Mean	76	46

Stem Inoculation Assay for Pathogenicity

Isolate Number	Recovered from Clone	Recovered at Site	Host Inoculated	Wound Appearance at 14 Days	Wound Size (mm2) at 40 Days
1461-10	NE 56	Milaca, MN	NE 299	Dark, sunken	160
1467-4	NE 252	Milaca, MN	DN 34	Dark, sunken	150
1481-7	NE 242	Mondovi, WI	DN 34	Brown, sunken	120
1467-4	NE 252	Milaca, MN	NE 299	Dark, swollen	120
1468-7	NE 54	Mondovi, WI	DN 34	Brown, callussing	119
1483-4	I 45/51	Milaca, MN	DN 34	Dark, open	105
1483-4	I 45/51	Milaca, MN	NE 299	Tan, callussing	100
1420-5	NE 252	Ashland, WI	NE 299	Tan, open	91
1468-7	NE 54	Mondovi, WI	NE 299	Brown, callussing	78
1439-4	Balsam poplar	Chippewa NF, MN	DN 34	Dark, sunken	72
1462-1	NE 28	Milaca, MN	DN 34	Clean, open	70
1481-7	NE 242	Mondovi, WI	NE 299	Tan, callussing	70
1461-10	NE 56	Milaca, MN	DN 34	Tan, sunken	66
1420-5	NE 252	Ashland, WI	DN 34	Brown, open	60
1480-4	NE 308	Mondovi, WI	NE 299	Clean, open	56
1471-1	DN 16	Milaca, MN	DN 34	Dark, sunken	55
1471-1	DN 16	Milaca, MN	NE 299	Clean, callussing	54
1439-4	Balsam poplar	Chippewa NF, MN	NE 299	Dark, open	50
Control	none	none	NE 299	Clean, callussing	48
1462-1	NE 28	Milaca, MN	NE 299	Brown, callussing	48
1480-4	NE 308	Mondovi, WI	DN 34	Dark, callussing	36
Control	none	none	DN 34	Clean, callussing	30

Amplification and Analysis of DNA from Septoria musiva and S. populicola Using the Polymerase Chain Reaction (PCR)

I. DNA Preparation

Fungal DNA is prepared according to Goodwin and Lee (1993). DNA concentration for each isolate is quantified using a Hoefer Fluorometer. Preparations are diluted to 1 $\mu g / \mu l$ DNA concentration.

II. Amplification

Buffer Solution	Per Reaction
BRL 10X buffer	1.5 μl
dNTP's mixture (2.5 mM ea. dATP, dGTP, dCTP, dTTP)	1.2
Operon primer	0.6
Bovine serum albumin	1.5
50 mM $MgCl^2$	0.6
Sterile, distilled water	6.95
Total	12.35 μl

	Per Reaction
Fungal DNA	2.5 μl

	Per Reaction
BRL Taq DNA polymerase	0.15 μl

Multiply amount of each component by the desired number of reactions. Combine components of buffer solution in a 2.5 ml microcentrifuge tube. Add fungal DNA to each well used in a 96-well thermocycler plate. Add 12.35 μl buffer solution to each well. Quickly add Taq. Layer each well with a drop of mineral oil. Place plate in Hybaid Omnigene Thermal Cycler for amplification. Cycling parameters used are 92.5°C for 1 1/2 min., 36°C for 2 min. and 72° C for 2 min., repeated for 45 cycles.

III. Analysis

Remove plate from cycler and add 3 μl 6X loading dye to each well. Electrophorese in an agarose gel (4.9 g. agarose in 350 ml .5X TBE buffer). Load a 10 kb DNA ladder in each gel row for later comparison. Stain gel in TBE with ethidium bromide for 25 minutes. Photograph gel under ultraviolet light.

Reference

Goodwin, D.C. and S.B. Lee. 1993. Microwave miniprep of total genomic DNA from fungi, plants, protists and animals for PCR. BioTechniques 15(3): 441-444.

Septoria Culture Collection as of March, 1995

Isolate	Host	Collection Site	Date	Number
SM1	Hybrid poplar	Ames, IA		1
SM3	Hybrid poplar	Ames, IA		1
488	Cottonwood	Rosemount, MN	Jul-84	1
513	Hybrid poplar	Rosemount, MN	May-86	1
602	P. trichocarpa	Puyallup, WA	Sep-88	1
605	Hybrid poplar	Rhinelander, WI	Sep-88	1
611	Hybrid poplar	Rosemount, MN	Oct-88	1
612	Hybrid poplar	Sioux Falls, SD	Oct-88	1
650	Hybrid poplar	Rhinelander, WI	Sep-89	1
652	Hybrid poplar	Puyallup, WA	Oct-89	1
698	Hybrid poplar	Milaca, MN	Aug-90	1
704	NE 300	Rhinelander, WI	Aug-90	1
706	NE 308	Ashland, WI	Aug-90	1
734	DN 65	Rosemount, MN	Jul-91	1
749	DTAC 8	Rosemount, MN	Jul-91	1
769	NE 27	Cloquet, MN	Aug-91	1
788	JACKII 4	Granite Falls, MN	Aug-91	1
794	NM 2	Milaca, MN	Aug-91	1
800	I45/51	Ashland, WI	Aug-91	1
900	JACKII 4	Milaca, MN	Jun-92	11
902	NE 242	Milaca, MN	Jun-92	6
903	DN 70	Milaca, MN	Jun-92	5
904	NE 351	Milaca, MN	Jun-92	2
905	DTAC 7	Milaca, MN	Jun-92	2
906	NE 54	Milaca, MN	Jun-92	1
910	NE 351	Milaca, MN	Jun-92	1
912	Aspen	Milaca, MN	Jun-92	1
913	NE 28	Milaca, MN	Jun-92	7
914	NM 2	Milaca, MN	Jun-92	5
915	JACKII 4	Mondovi, WI	Jun-92	1
916	NE 351	Mondovi, WI	Jun-92	3
917	NE 54	Mondovi, WI	Jun-92	3
918	NE 56	Mondovi, WI	Jun-92	4
919	NE 242	Mondovi, WI	Jun-92	1
920	I45-1	Mondovi, WI	Jun-92	1
922	Balsam poplar	Cloquet, MN	Jun-92	5
923	DTAC 10	Rosemount, MN	Jun-92	6
924	DTAC 9	Rosemount, MN	Jul-92	3
925	DTAC 7	Milaca, MN	Jul-92	4
926	DN 93	Milaca, MN	Jul-92	2
928	DN 30	Milaca, MN	Jul-92	1
929	NE 351	Milaca, MN	Jul-92	1
930	NM 2	Milaca, MN	Jul-92	3

Isolate	Host	Collection Site	Date	Number
931	NM 6	Milaca, MN	Jul-92	12
933	NE 28	Milaca, MN	Jul-92	2
934	NE 351	Milaca, MN	Jul-92	1
937	I45/51	Milaca, MN	Jul-92	6
938	NE 308	Milaca, MN	Jul-92	2
939	NE 242	Milaca, MN	Jul-92	1
940	NE 299	Milaca, MN	Jul-92	2
941	DN 70	Milaca, MN	Jul-92	8
942	DN 16	Milaca, MN	Jul-92	2
943	NE 56	Milaca, MN	Jul-92	2
945	JACKII 4	Milaca, MN	Jul-92	4
946	NE 242	Milaca, MN	Jul-92	5
947	NM2	Milaca, MN	Jul-92	17
948	JACKII 4	Mondovi, WI	Jul-92	1
949	NE 242	Mondovi, WI	Jul-92	3
950	I45/51	Mondovi, WI	Jul-92	3
951	DTAC 16	Mondovi, WI	Jul-92	1
952	NE 56	Mondovi, WI	Jul-92	2
955	Balsam poplar	Rhinelander, WI	Aug-92	1
956	P. trichocarpa	Rhinelander, WI	Aug-92	3
957	Balsam poplar	Rhinelander, WI	Aug-92	9
958	P. trichocarpa	Rhinelander, WI	Aug-92	4
959	P. trichocarpa	Rhinelander, WI	Aug-92	1
960	Balsam poplar	Northome, MN	Aug-92	1
961	Aspen	Grand Rapids, MN	Aug-92	2
962	Balsam poplar	Grand Rapids, MN	Aug-92	7
963	Aspen	Int'l Falls, MN	Aug-92	1
967	NM 6	Milaca, MN	Aug-92	2
970	NE 50	Milaca, MN	Aug-92	1
971	I45/51	Milaca, MN	Aug-92	1
972	NM 2	Milaca, MN	Aug-92	8
973	NE 242	Milaca, MN	Aug-92	6
974	DN 16	Milaca, MN	Aug-92	1
975	DTAC 16	Mondovi, WI	Aug-92	3
977	JACKII 4	Mondovi, WI	Aug-92	1
979	DTAC 26	Mondovi, WI	Aug-92	4
980	NE 351	Mondovi, WI	Aug-92	2
981	NE 242	Mondovi, WI	Aug-92	1
983	NE 56	Mondovi, WI	Aug-92	2
985	I45/51	Fairmont, MN	Aug-92	1
986	NM 6	Fairmont, MN	Aug-92	3
992	Aspen	Milaca, MN	Sep-92	1
994	DN 34	Milaca, MN	Sep-92	1
997	DTAC 16	Milaca, MN	Sep-92	4
998	I45/51	Milaca, MN	Sep-92	2

Isolate	Host	Collection Site	Date	Number
999	I45-1	Milaca, MN	Sep-92	3
1000	DN 131	Milaca, MN	Sep-92	3
1003	DN 19	Fargo, ND	Sep-92	1
1004	NE 19	Fargo, ND	Sep-92	9
1005	NM 2	Fargo, ND	Sep-92	2
1006	DN 55	Fargo, ND	Sep-92	4
1007	Cottonwood	Fargo, ND	Sep-92	6
1008	JACKII 4	Fargo, ND	Sep-92	1
1009	NE 252	Ashland, WI	Sep-92	4
1010	Siouxland	Ashland, WI	Sep-92	8
1011	DN 34	Ashland, WI	Sep-92	7
1013	NE 258	Ashland, WI	Sep-92	5
1014	NE 308	Ashland, WI	Sep-92	2
1015	DTAC 26	Ashland, WI	Sep-92	8
1016	NE 299	Ashland, WI	Sep-92	5
1017	NM 6	Ashland, WI	Sep-92	7
1018	DN 174	Ashland, WI	Sep-92	1
1019	NM 2	Ashland, WI	Sep-92	7
1020	P. alba	Ashland, WI	Sep-92	7
1021	NE 19	Ashland, WI	Sep-92	10
1022	DN 182	Granite Falls, MN	Sep-92	2
1023	NE 308	Granite Falls, MN	Sep-92	1
1024	DN 34	Granite Falls, MN	Sep-92	3
1025	DN 17	Granite Falls, MN	Sep-92	2
1026	NE 17	Granite Falls, MN	Sep-92	2
1027	Siouxland	Granite Falls, MN	Sep-92	3
1028	NE 47	Cloquet, MN	Sep-92	3
1031	NE 51	Cloquet, MN	Sep-92	1
1033	NE 27	Cloquet, MN	Sep-92	4
1034	NE 41	Cloquet, MN	Sep-92	1
1035	NE 28	Cloquet, MN	Sep-92	2
1036	DTAC 26	Rhinelander, WI	Sep-92	2
1038	DN 160	Rhinelander, WI	Sep-92	1
1039	NM 6	Rhinelander, WI	Sep-92	8
1040	DTAC 16	Rhinelander, WI	Sep-92	3
1041	NC 5260	Rhinelander, WI	Sep-92	3
1043	NM 2	Rhinelander, WI	Sep-92	1
1044	DTAC 4	Ontario, Can.	Sep-92	1
1045	NE 351	Milaca, MN	Sep-92	2
1046	DTAC 7	Milaca, MN	Sep-92	5
1047	JACKII 4	Milaca, MN	Sep-92	2
1048	NM 6	Milaca, MN	Sep-92	10
1049	DN 16	Milaca, MN	Sep-92	7
1050	NE 308	Milaca, MN	Sep-92	8
1051	DN 70	Milaca, MN	Sep-92	5

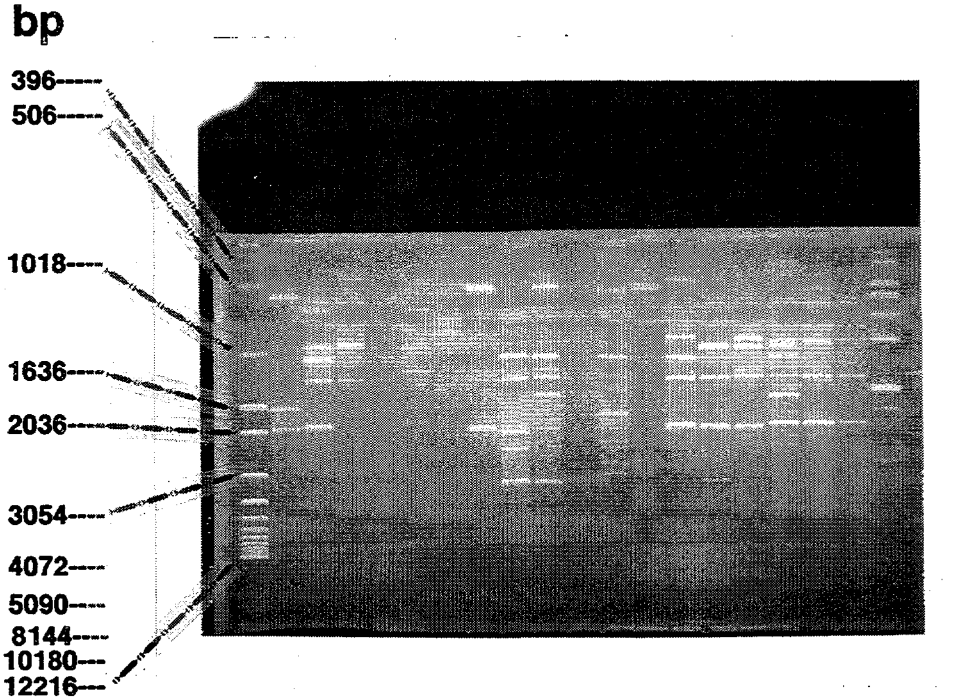
Isolate	Host	Collection Site	Date	Number
1052	NE 50	Milaca, MN	Sep-92	3
1053	NM 2	Milaca, MN	Sep-92	4
1054	NE 28	Milaca, MN	Sep-92	3
1055	I45/51	Milaca, MN	Sep-92	3
1057	NE 299 Somaclone	Rhinelander, WI	Oct-92	2
1059	NE 299 Somaclone	Rhinelander, WI	Oct-92	1
1060	NE 299 Somaclone	Rhinelander, WI	Oct-92	2
1063	NE 299 Somaclone	Rhinelander, WI	Oct-92	1
1064	DTAC 16	Milaca, MN	Jul-93	1
1066	NE 252	Milaca, MN	Jul-93	1
1067	NE 351	Milaca, MN	Jul-93	3
1068	NE 351	Milaca, MN	Jul-93	1
1069	DN 34	Milaca, MN	Jul-93	6
1070	NM 2	Milaca, MN	Jul-93	3
1071	NE 242	Milaca, MN	Jul-93	2
1072	JACKII 4	Milaca, MN	Jul-93	2
1073	NE 308	Milaca, MN	Jul-93	3
1074	DN 70	Milaca, MN	Jul-93	1
1076	NM 6	Milaca, MN	Jul-93	7
1078	I 45/51	Milaca, MN	Jul-93	2
1079	DN 16	Milaca, MN	Jul-93	2
1080	NE 56	Milaca, MN	Jul-93	1
1083	NM 2	Mondovi, WI	Jul-93	5
1085	NE 351	Mondovi, WI	Jul-93	3
1086	NE 252	Mondovi, WI	Jul-93	3
1087	DTAC 26	Mondovi, WI	Jul-93	2
1088	NE 242	Mondovi, WI	Jul-93	5
1089	NM 6	Mondovi, WI	Jul-93	3
1090	NE 299	Mondovi, WI	Jul-93	4
1096	NE 54	Mondovi, WI	Aug-93	1
1097	NM 2	Mondovi, WI	Aug-93	1
1099	NE 242	Mondovi, WI	Aug-93	1
1102	NE 299	Mondovi, WI	Aug-93	2
1105	NE 351	Mondovi, WI	Aug-93	2
1106	NE 56	Mondovi, WI	Aug-93	2
1107	NE 252	Mondovi, WI	Aug-93	1
1110	Triploid aspen	Mondovi, WI	Aug-93	4
1112	DTAC 26	Milaca, MN	Aug-93	2
1113	NM 2	Milaca, MN	Aug-93	3
1114	NM 6	Milaca, MN	Aug-93	4
1115	DN 16	Milaca, MN	Aug-93	1
1116	DTAC 7	Milaca, MN	Aug-93	2
1117	JACKII 4	Milaca, MN	Aug-93	2
1118	NE 56	Milaca, MN	Aug-93	8
1120	DTAC 16	Milaca, MN	Aug-93	2

Isolate	Host	Collection Site	Date	Number
1121	NE 299	Milaca, MN	Aug-93	1
1122	NE 252	Milaca, MN	Aug-93	2
1123	NE 308	Milaca, MN	Aug-93	3
1124	NE 54	Milaca, MN	Aug-93	4
1126	NE 28	Milaca, MN	Aug-93	1
1128	NE 51	Milaca, MN	Aug-93	2
1131	Balsam poplar	St. Paul, MN	Aug-93	7
1135	Cottonwood	Rosemount, MN	Aug-93	2
1136	Cottonwood	Rosemount, MN	Aug-93	1
1137	Triploid aspen	Rosemount, MN	Aug-93	2
1138	Aspen	Rosemount, MN	Aug-93	2
1139	JACKII 4	Rhinelander, WI	Sep-93	2
1140	NC 5260	Rhinelander, WI	Sep-93	4
1141	DTAC 26	Rhinelander, WI	Sep-93	1
1142	NE 299	Rhinelander, WI	Sep-93	2
1143	NE 242	Rhinelander, WI	Sep-93	4
1144	NE 351	Rhinelander, WI	Sep-93	1
1145	NE 252	Rhinelander, WI	Sep-93	2
1146	NM 2	Rhinelander, WI	Sep-93	4
1147	NE 308	Rhinelander, WI	Sep-93	1
1149	NM 2	Fargo, ND	Sep-93	8
1150	DN 74	Fargo, ND	Sep-93	8
1151	Cottonwood	Fargo, ND	Sep-93	5
1152	Cottonwood	Granite Falls, MN	Sep-93	1
1153	NM 6	Granite Falls, MN	Sep-93	1
1154	JACKII 4	Fargo, ND	Sep-93	3
1155	I 45/51	Fargo, ND	Sep-93	2
1156	NM 6	Sioux Falls, SD	Sep-93	2
1157	DN 34	Granite Falls, MN	Sep-93	1
1158	NE 308	Granite Falls, MN	Sep-93	5
1159	JACKII 4	Granite Falls, MN	Sep-93	4
1160	NM 2	Granite Falls, MN	Sep-93	6
1161	NC 5260	Ashland, WI	Sep-93	4
1162	NE 351	Ashland, WI	Sep-93	2
1163	NE 54	Sioux Falls, SD	Sep-93	1
1165	NE 242	Sioux Falls, SD	Sep-93	2
1167	NM 6	Sioux Falls, SD	Sep-93	2
1168	DN 34	Sioux Falls, SD	Sep-93	3
1169	DTAC 16	Sioux Falls, SD	Sep-93	1
1171	DTAC 7	Fairmont, MN	Sep-93	2
1172	NC 5260	Fairmont, MN	Sep-93	1
1174	DTAC 16	Ashland, WI	Sep-93	2
1175	NE 242	Ashland, WI	Sep-93	2
1178	NE 308	Ashland, WI	Sep-93	1
1179	NC 5260	Fargo, ND	Sep-93	7

Isolate	Host	Collection Site	Date	Number
1181	DN 34	Ashland, WI	Sep-93	1
1182	DN 70	Granite Falls, MN	Sep-93	6
1183	DTAC 7	Granite Falls, MN	Sep-93	2
1184	P. trichocarpa	West Coast	Sep-93	2
1187	P. trichocarpa	West Coast	Sep-93	1
1188	P. trichocarpa	West Coast	Sep-93	4
1189	P. trichocarpa	West Coast	Sep-93	1
1193	P. trichocarpa	West Coast	Sep-93	2
1197	P. trichocarpa	West Coast	Sep-93	1
1198	P. trichocarpa	West Coast	Sep-93	1
1199	P. trichocarpa	West Coast	Sep-93	3
1400	P. trichocarpa	West Coast	Sep-93	1
1401	P. trichocarpa	West Coast	Sep-93	2
1404	P. trichocarpa	West Coast	Sep-93	2
1405	P. trichocarpa	West Coast	Sep-93	2
1406	Balsam Poplar	Cloquet, MN	Sep-93	2
1414	NE 41	Ashland, WI	Sep-93	2
1418	NE 17	Sioux Falls, SD	Sep-93	2
1420	NE 252	Ashland, WI	Sep-93	5
1427	P. trichocarpa	West Coast	Sep-93	1
1430	NM 2	Mondovi, WI	Sep-93	2
1431	Diploid aspen	Milaca, MN	Sep-93	1
1432	JACKII 4	Milaca, MN	Sep-93	2
1433	Aspen	Milaca, MN	Sep-93	1
1435	DTAC 26	Milaca, MN	Sep-93	4
1437	NM 2	Milaca, MN	Sep-93	2
1438	NE 54	Milaca, MN	Sep-93	5
1439	Balsam Poplar	Chippewa NF	Sep-93	2
1440	NE 308	Milaca, MN	Sep-93	1
1441	NE 28	Mondovi, WI	Sep-93	1
1442	NM 6	Milaca, MN	Sep-93	2
1444	P. trichocarpa	West Coast	Sep-93	2
1445	P. trichocarpa	West Coast	Sep-93	1
1446	P. trichocarpa	West Coast	Sep-93	2
1447	P. trichocarpa	West Coast	Sep-93	3
1450	P. trichocarpa	West Coast	Sep-93	4
1451	P. trichocarpa	West Coast	Sep-93	2
1452	P. trichocarpa	West Coast	Sep-93	3
1453	P. trichocarpa	West Coast	Sep-93	2
1455	P. trichocarpa	West Coast	Sep-93	1
1457	P. trichocarpa	West Coast	Sep-93	3
1460	P. trichocarpa	West Coast	Sep-93	4
1461	NE 56	Milaca, MN	Sep-93	4
1462	NE 28	Milaca, MN	Sep-93	1
1467	NE 252	Milaca, MN	Sep-93	1

Isolate	Host	Collection Site	Date	Number
1468	NE 54	Mondovi, WI	Sep-93	4
1469	NE 299	Milaca, MN	Sep-93	3
1473	NE 252	Mondovi, WI	Sep-93	1
1475	DTAC 26	Milaca, MN	Sep-93	1
1476	DN 34	Milaca, MN	Sep-93	1
1478	NE 56	Mondovi, WI	Sep-93	1
1479	NE 351	Milaca, MN	Sep-93	1
1480	NE 308	Mondovi, WI	Sep-93	5
1481	NE 242	Mondovi, WI	Sep-93	2
1482	NE 351	Mondovi, WI	Sep-93	1
1483	I 45/51	Milaca, MN	Sep-93	5
1485	DTAC 16	Milaca, MN	Aug-94	3
1486	Balsam poplar	Milaca, MN	Aug-94	3
1489	DN 70	Milaca, MN	Aug-94	1
1490	NE 242	Milaca, MN	Aug-94	1
1491	DN 16	Milaca, MN	Aug-94	1
1495	NM 6	Milaca, MN	Aug-94	3
1496	NE 308	Milaca, MN	Aug-94	4
1497	NM 2	Milaca, MN	Aug-94	2
1499	NM 2	Mondovi, WI	Sep-94	5
1507	DN 16	Mondovi, WI	Sep-94	2
1508	NE 351	Mondovi, WI	Sep-94	1
1509	NE 308	Mondovi, WI	Sep-94	1
1510	NE 56	Mondovi, WI	Sep-94	1
1511	Balsam poplar	Cloquet, MN	Sep-94	3
1512	Cottonwood	Savage, MN	Sep-94	7
1513	Aspen	Cloquet, MN	Sep-94	5
1514	Aspen	Rosemount, MN	Sep-94	7
1515	Cottonwood	Rosemount, MN	Sep-94	12
1516	Balsam poplar	Rosemount, MN	Sep-94	1
1519	47-174	Rosemount, MN	Sep-94	1
1523	Balsam poplar	Cloquet, MN	Sep-94	3
			Total	859

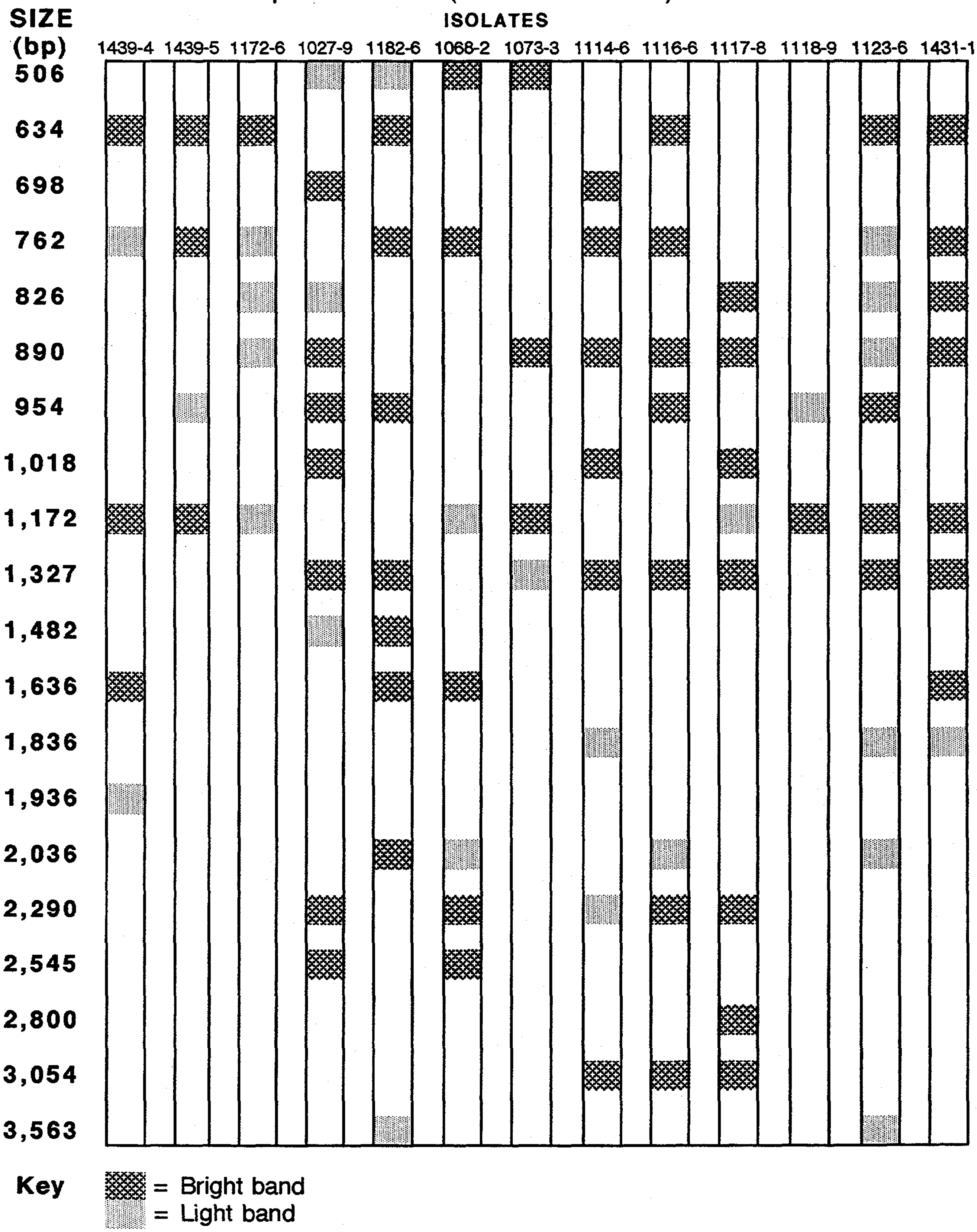
RAPD Analysis of 17 S. musiva isolates

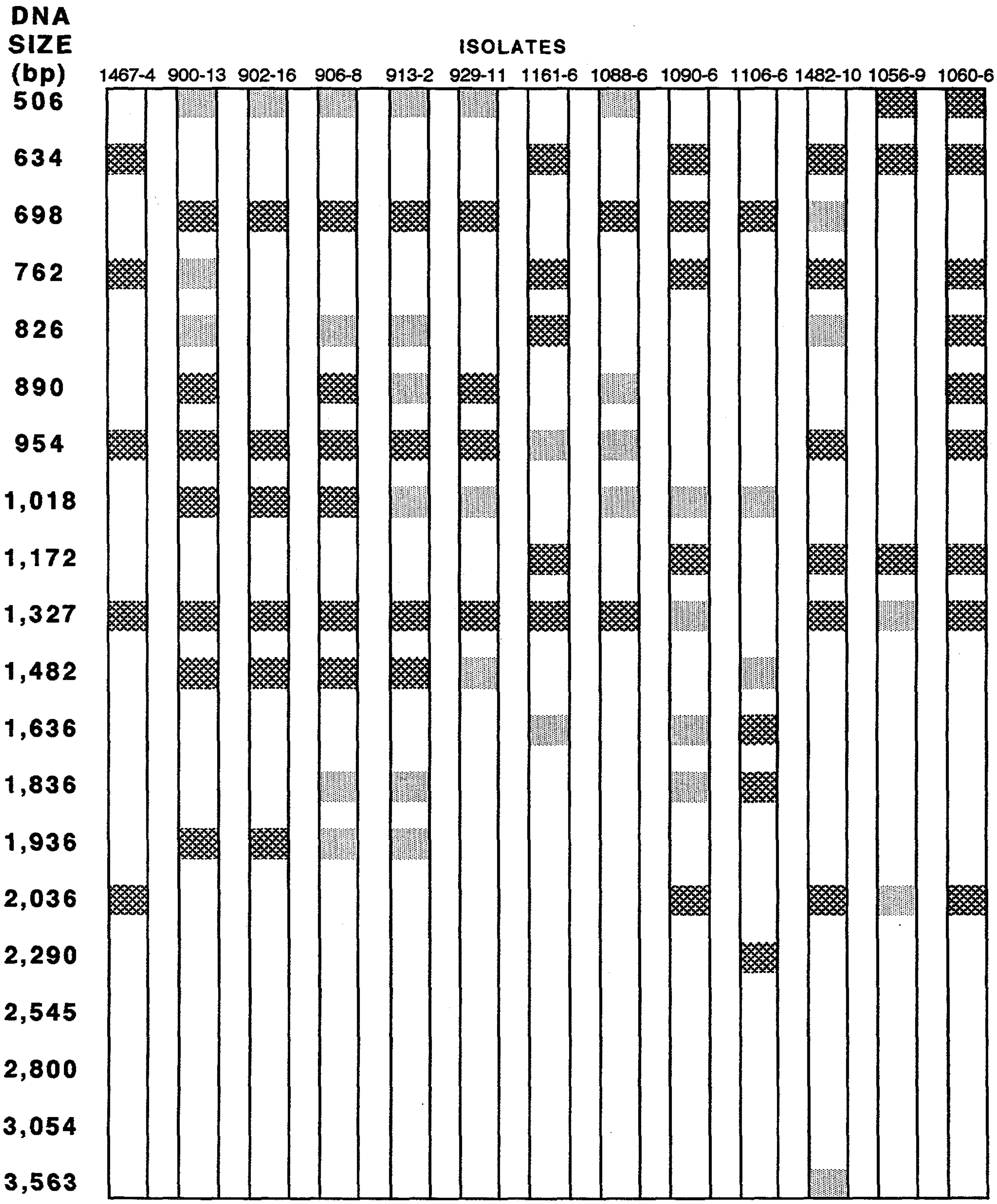


1. 10 kb ladder
2. 1035-2, NE 28, Cloquet, MN
3. 1041-5, NC 5260, Rhinelander, WI
4. 1043-1, NM 2, Rhinelander, WI
5. 1045-11, NE 351, Milaca, MN
6. 1046-19, DTAC 7, Milaca, MN
7. 1047-13, Jackii 4, Milaca, MN
8. 1048-5, NM 6, Milaca, MN
9. 1049-8, DN 16, Milaca, MN
10. 1050-2, NE 308, Milaca, MN
11. 1052-5, NE 50, Milaca, MN
12. 1067-5, NE 351, Milaca, MN
13. 1069-1, DN 34, Milaca, MN
14. 1078-9, I 45/51, Milaca, MN
15. 1079-8, DN 16, Milaca, MN
16. 1083-6, NM 2, Mondovi, MN
17. 1086-10, NE 252, Milaca, MN
18. 1088-2, NE 242, Mondovi, WI
19. non-Septoria control
20. non-Septoria control

Results of RAPD Analyses of Septoria Isolates

Operon Primer 10 (5'GGAAGCTTGG3')

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1467-4
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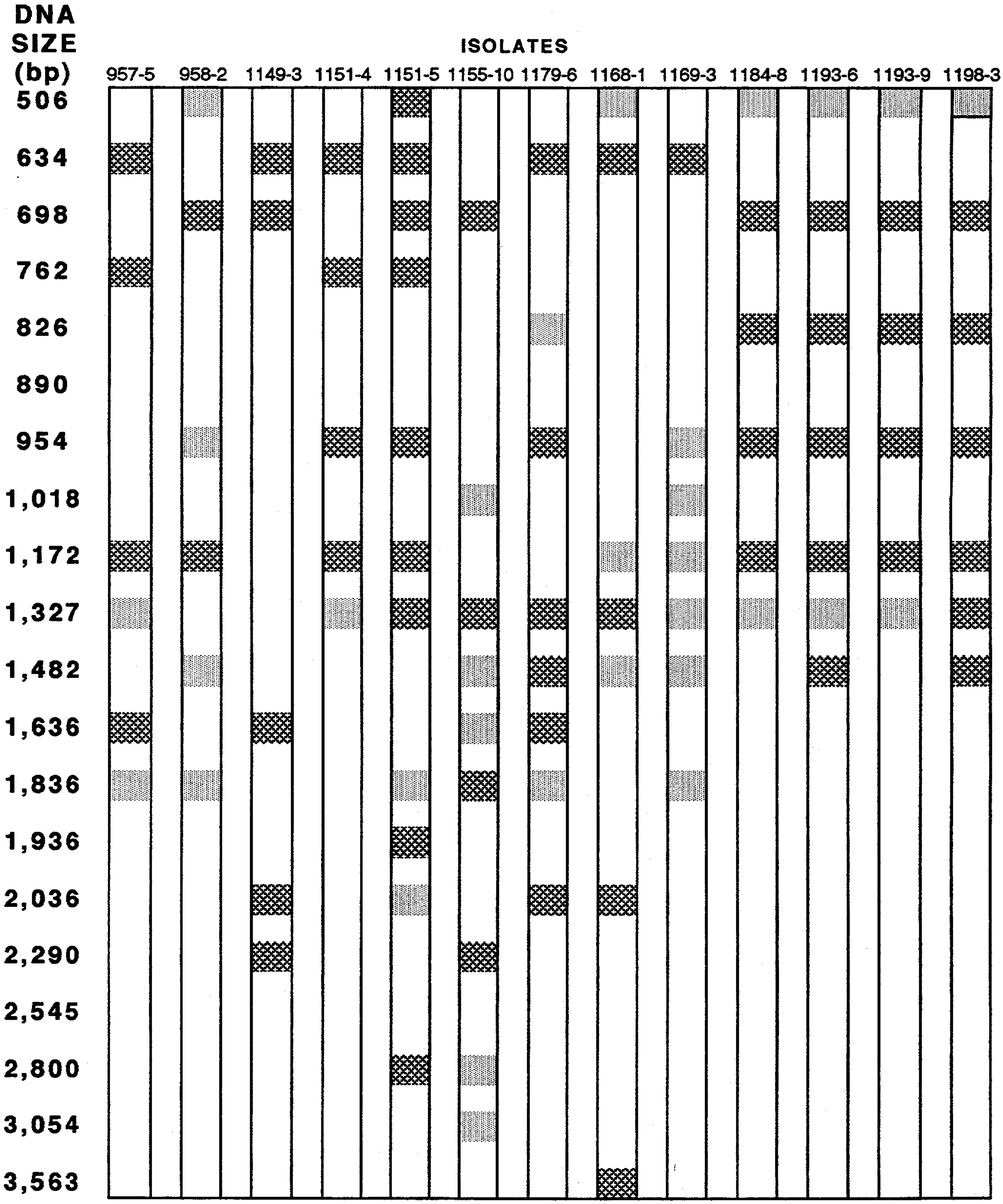
902-16
906-8
913-2
929-11
1161-6
1088-6
1090-6
1106-6
1482-10
1056-9
1060-6
506
634
698
762
826
890
954
1,018
1,172
1,327
1,482
1,636
1,836
1,936
2,036
2,290
2,545
2,800
3,054
3,563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957-5
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1155-10
1179-6
1168-1
1169-3
1184-8
1193-6
1193-9
1198-3
506
634
698
762
826
890
954
1,018
1,172
1,327
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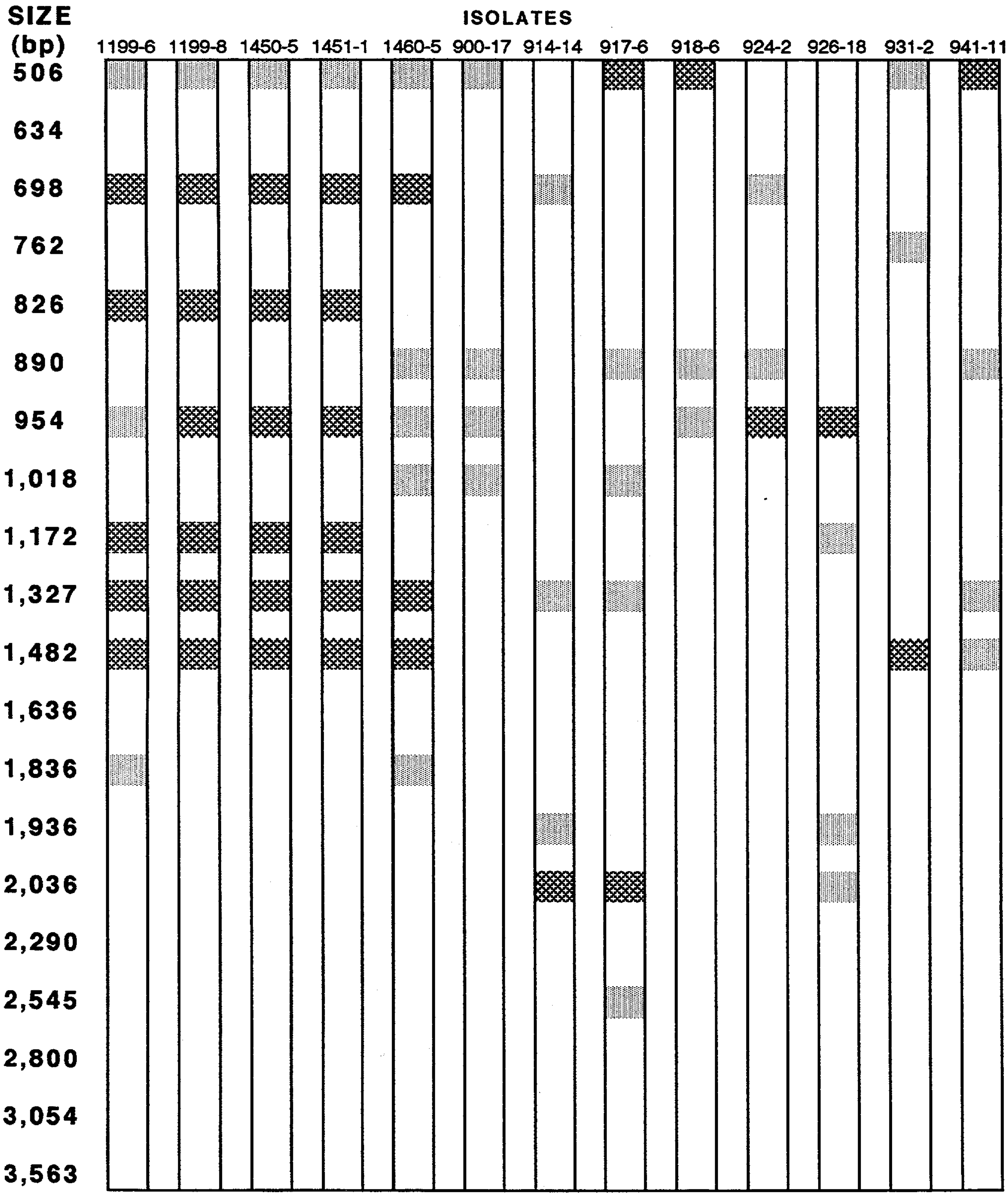
1,636
1,836
1,936
2,036
2,290
2,545
2,800
3,054
3,563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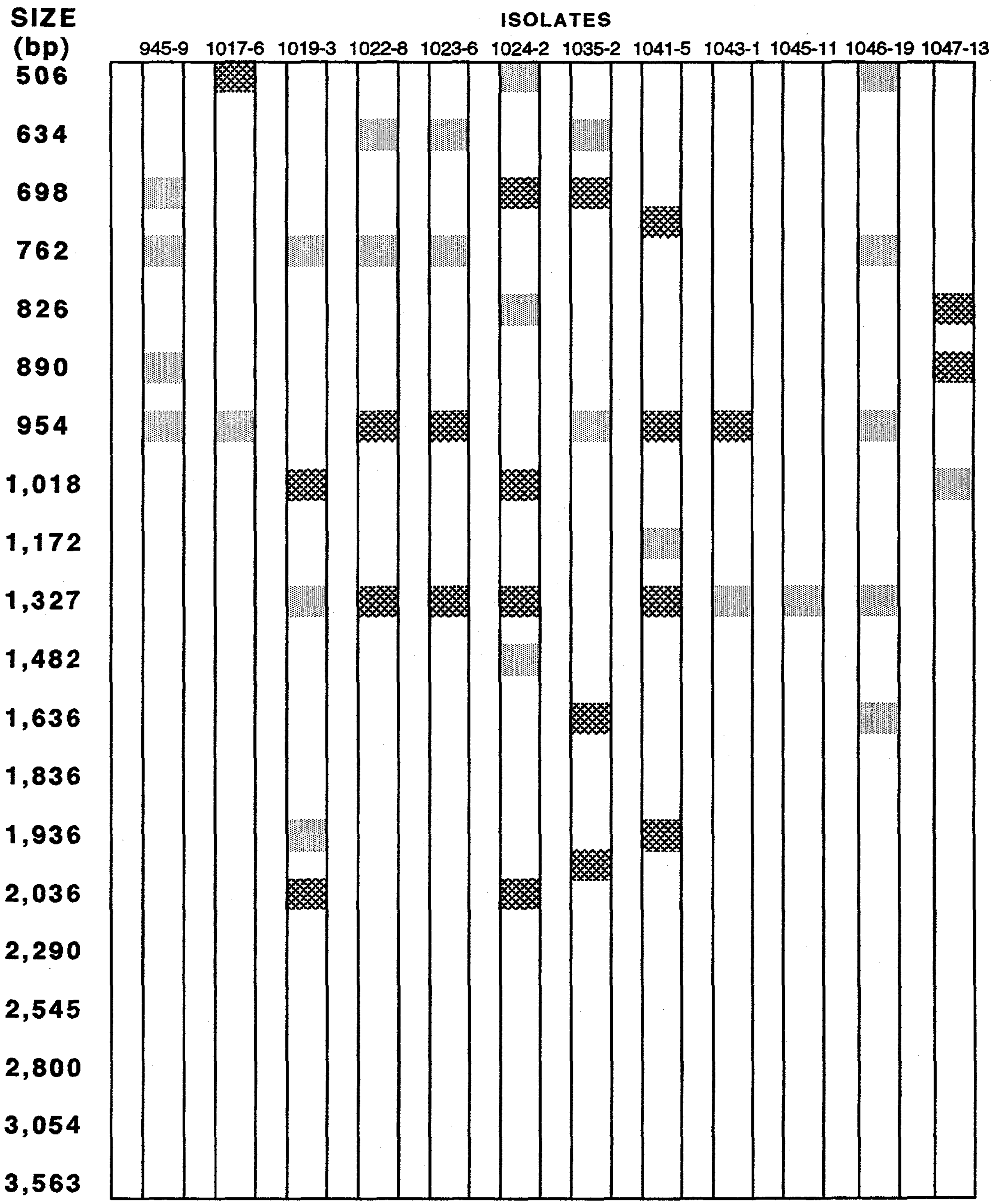
900-17
914-14
917-6
918-6
924-2
926-18
931-2
941-11
506
634
698
762
826
890
954
1,018
1,172
1,327
1,482
1,636
1,836
1,936
2,036
2,290
2,545
2,800
3,054
3,563


SIZE (bp)
ISOLATES
945-9 1017-6 1019-3 1022-8 1023-6 1024-2 1035-2 1041-5 1043-1 1045-11 1046-19 1047-13
506
634
698
762
826
890
954
1,018
1,172
1,327
1,482
1,636
1,836
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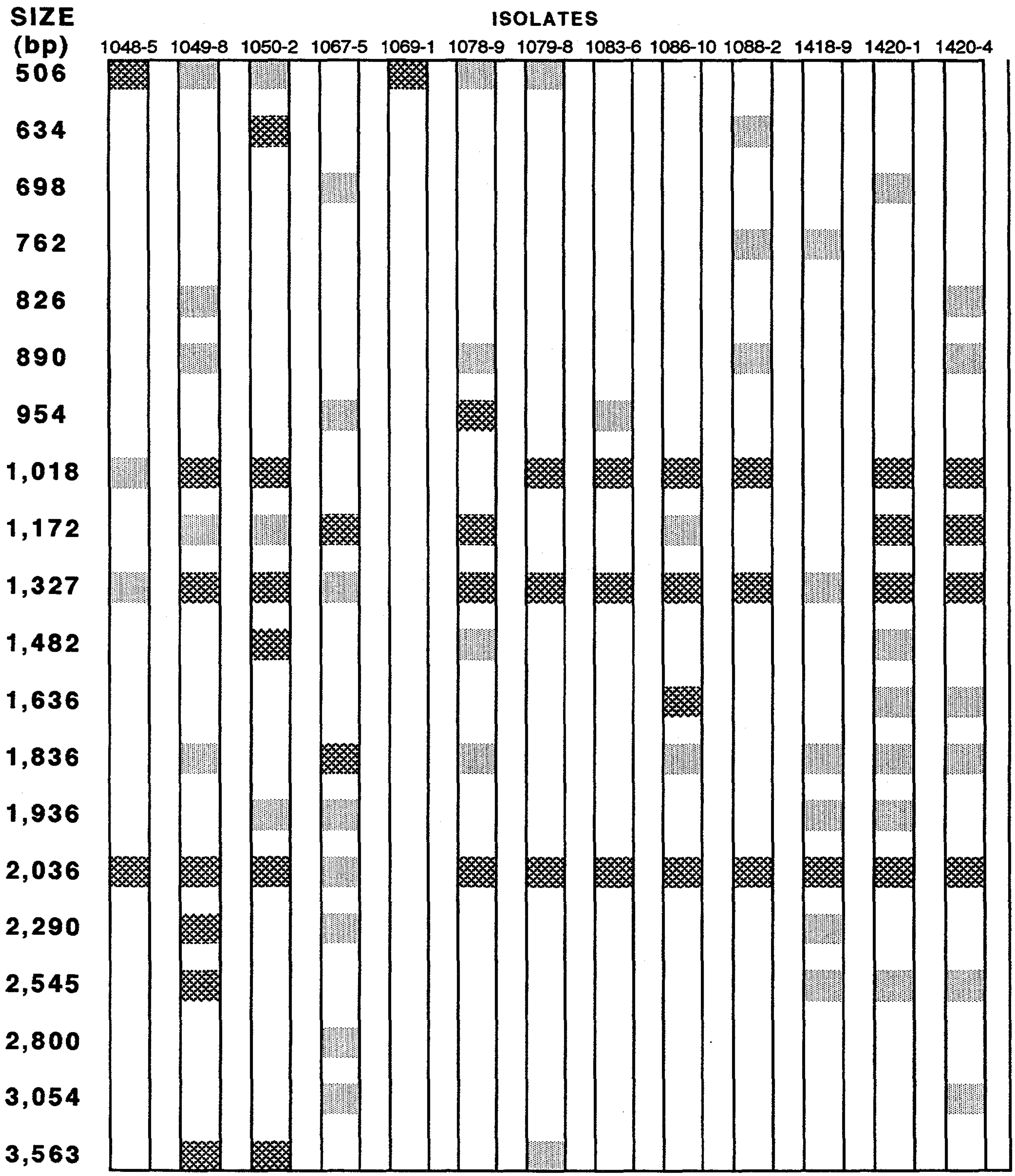
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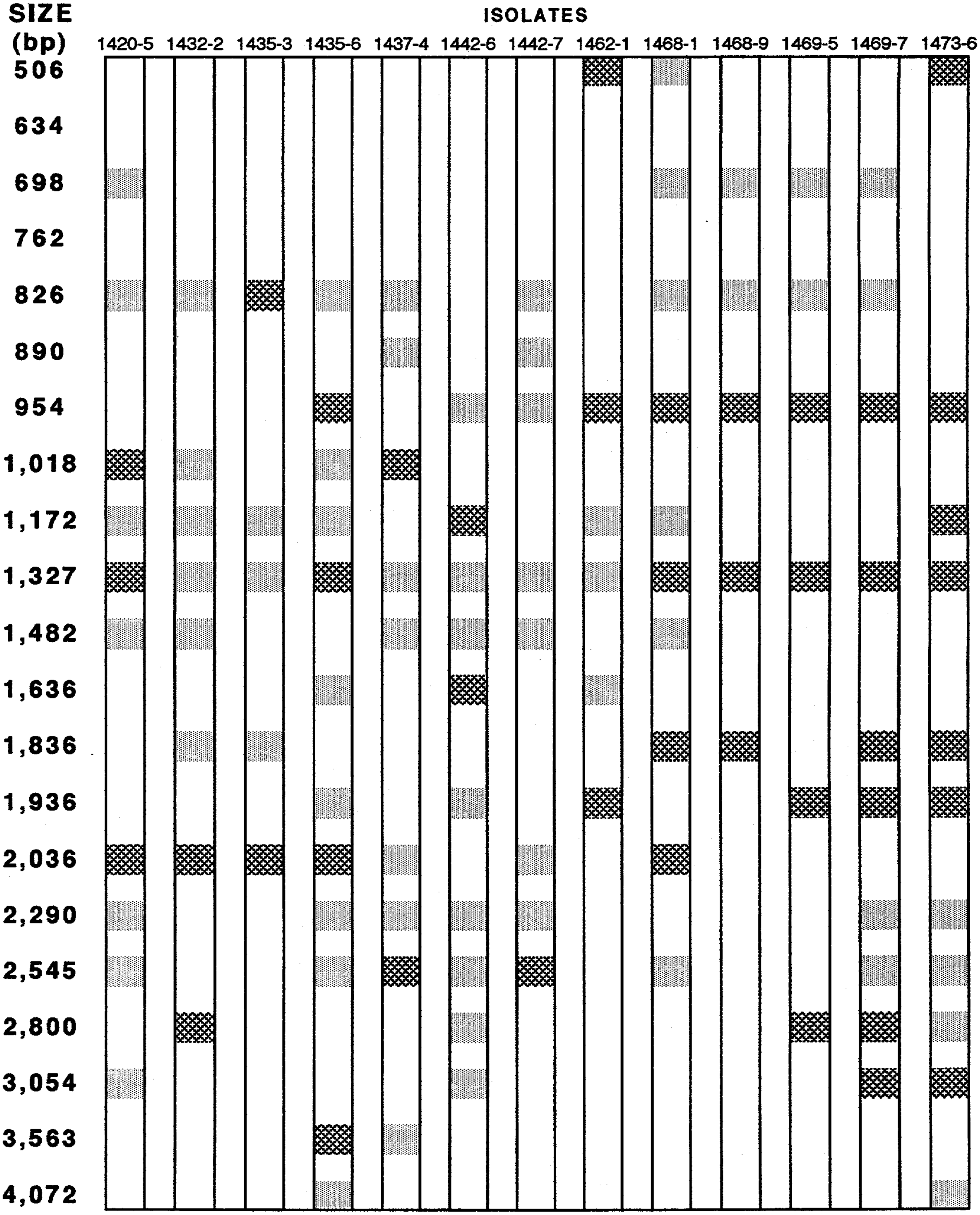

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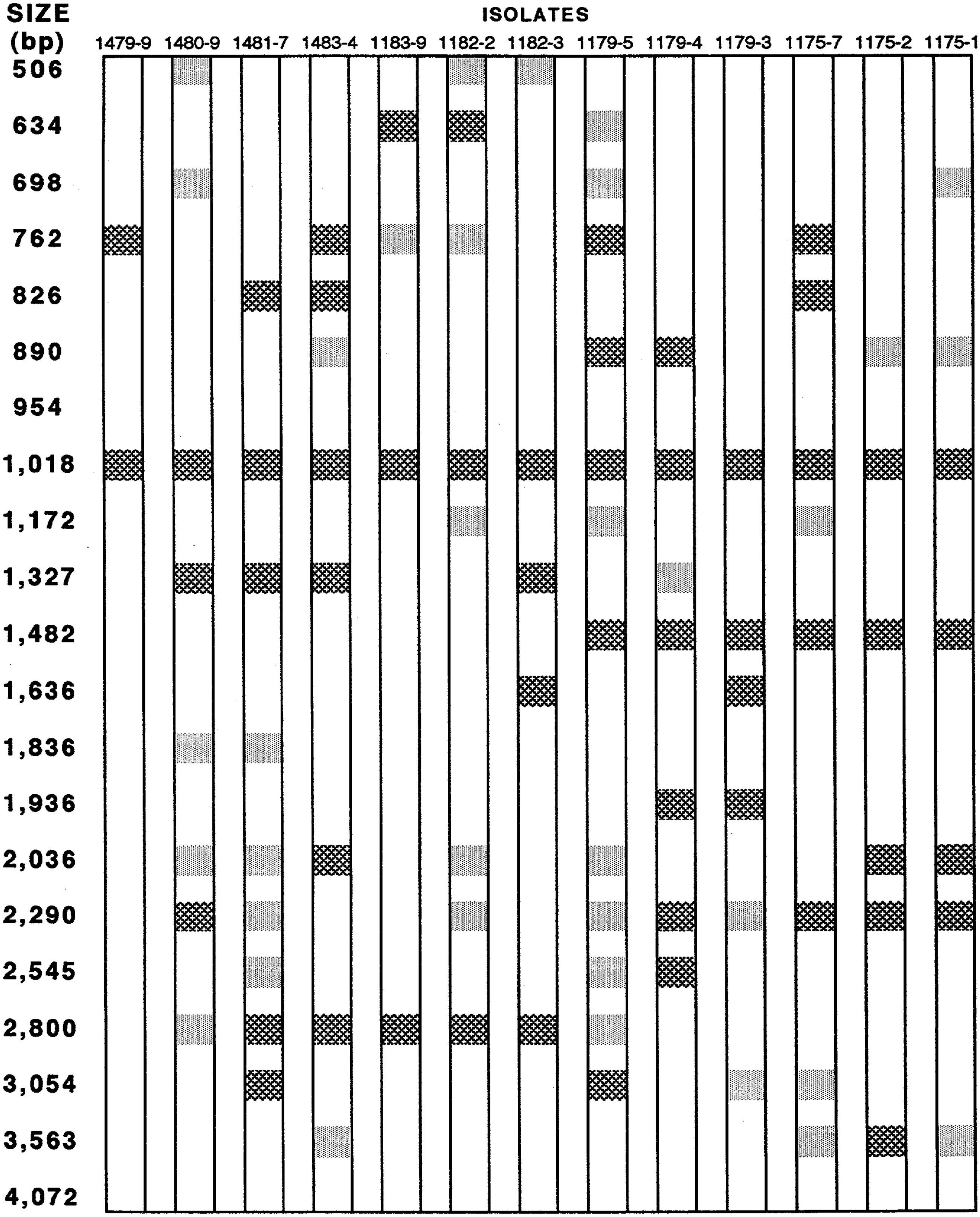
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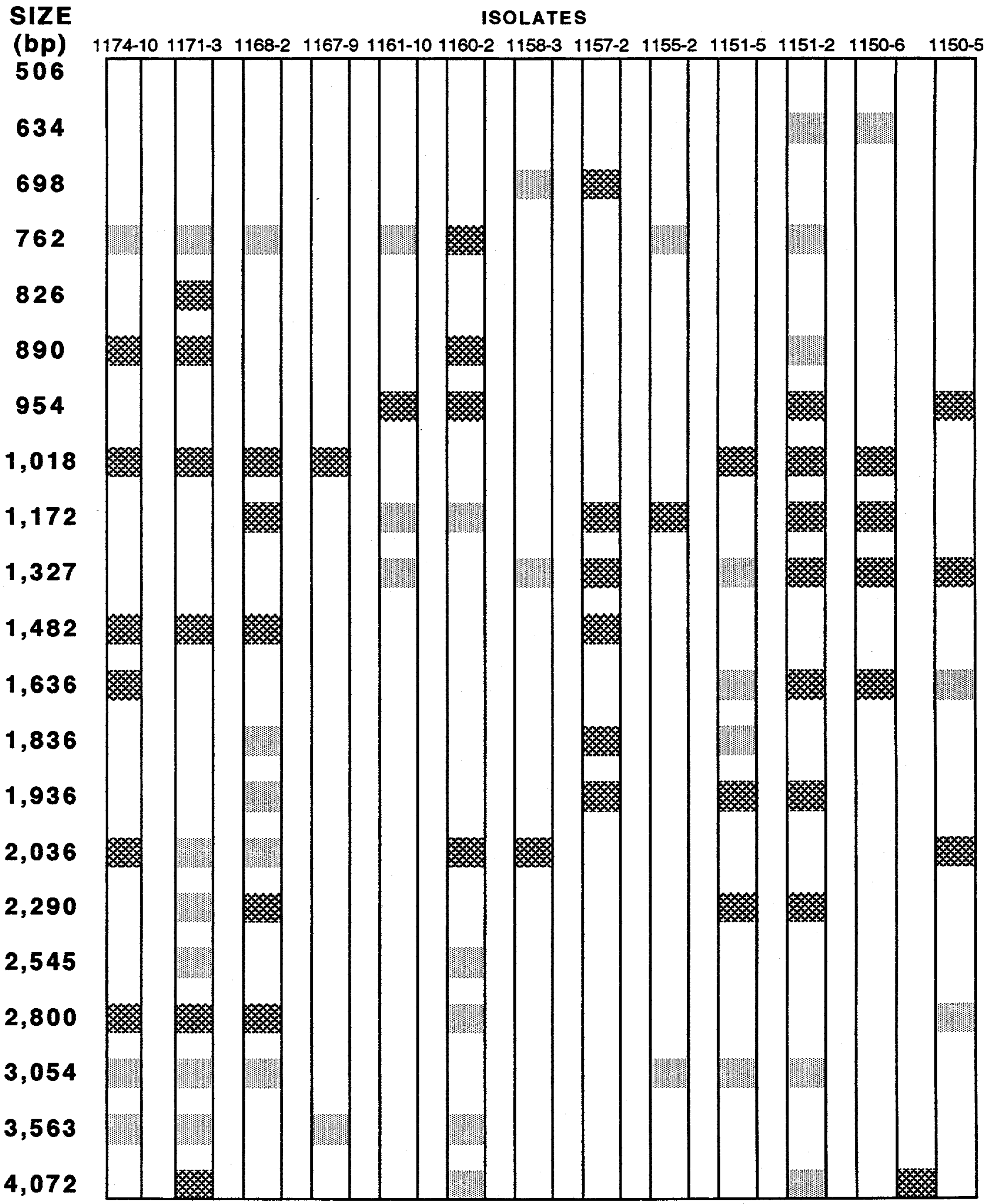
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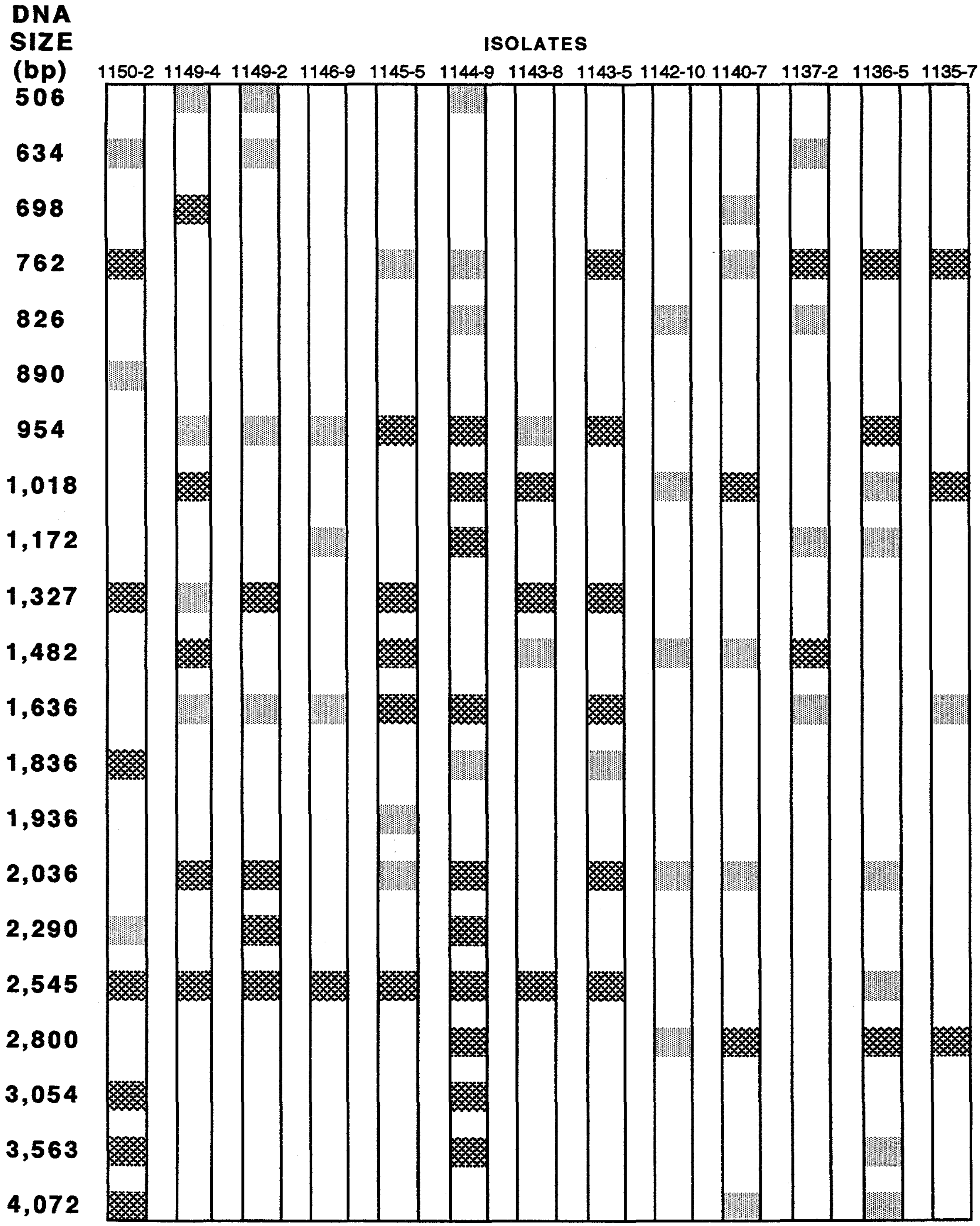
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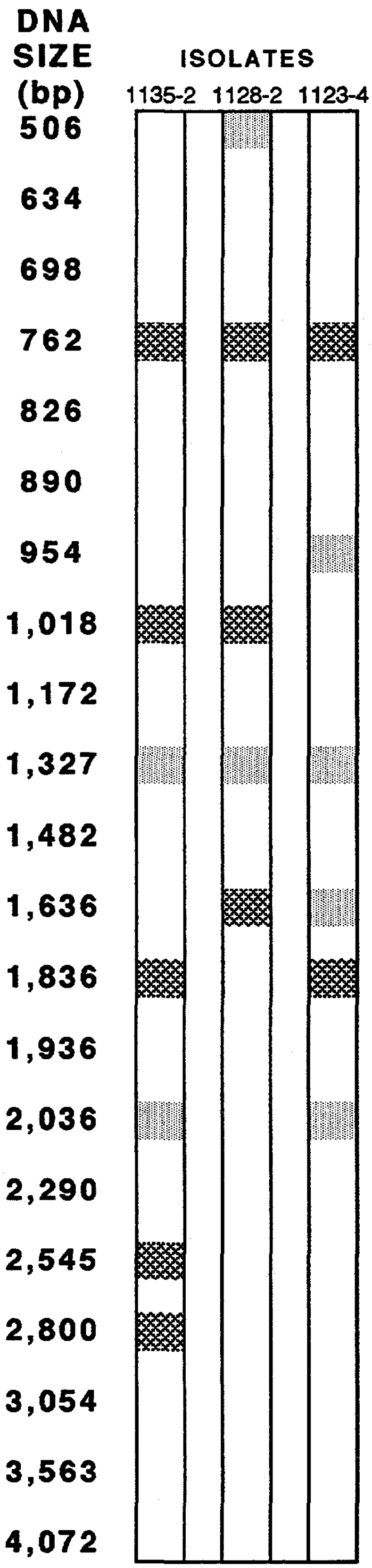
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Example of Results from Leaf Disk Bioassay for Septoria Leaf Spot Resistance of NE 308

(% green leaf area remaining after inoculation)

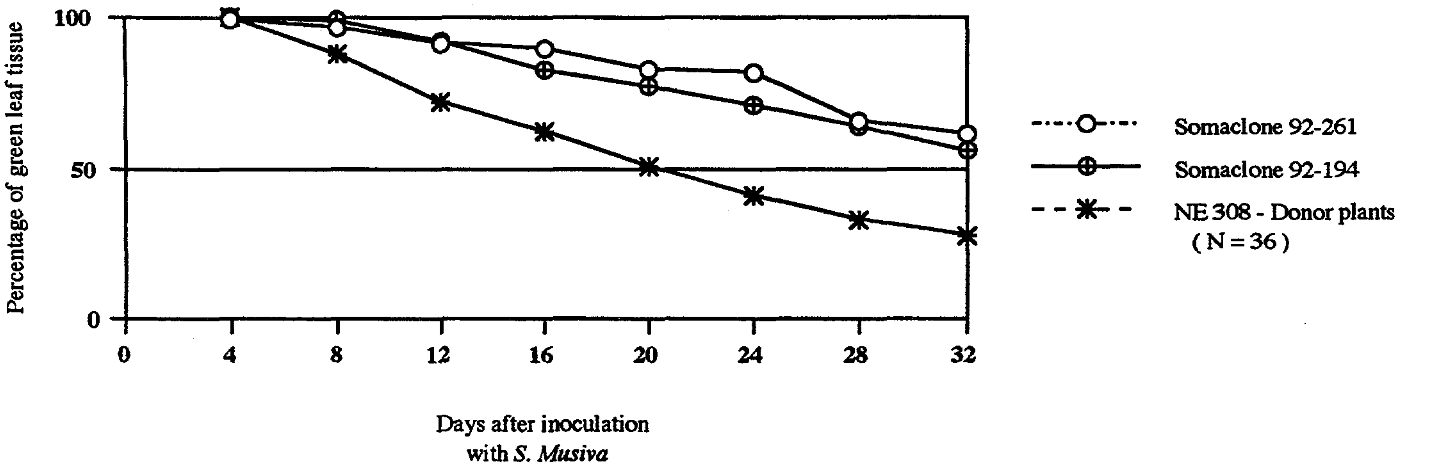
TRIAL #	DAY 4	DAY 8	DAY 12	DAY 16	DAY 20	DAY 24	DAY 28	DAY 32
193	100	92	76	69	51	32	32	21
196	98	35	22	9	7	5	4	2
197	100	100	77	67	68	60	54	45
198	100	99	76	59	51	46	39	34
199	99	99	93	86	80	72	59	54
200	100	87	74	50	37	29	28	27
201	100	98	94	83	83	82	76	75
202	100	100	97	94	65	37	33	27
203	100	100	99	96	71	64	51	47
204	99	99	87	83	52	58	35	22
205	100	58	23	10	3	1	0	0
206	100	100	98	92	87	76	66	50
207	100	89	77	52	42	36	11	7
208	100	98	87	59	31	7	1	0
209	100	72	47	39	38	27	28	21
210	100	97	95	90	76	66	49	43
211	99	89	70	56	49	43	38	32
212	99	95	93	92	84	65	55	50
213	99	91	57	44	46	43	38	39
214	100	84	60	61	53	44	37	35
215	99	68	48	47	36	16	22	17
216	100	97	90	86	72	64	59	55
217	100	96	91	90	81	74	67	48
218	100	56	39	31	25	17	14	10
219	100	93	74	69	61	56	47	43
220	97	97	72	54	47	43	27	26
221	99	94	62	37	16	0	0	0
222	99	87	61	50	28	4	1	0
224	98	87	78	68	52	49	40	37
225	100	99	96	94	92	87	75	58
226	98	90	81	70	45	28	16	14
227	98	79	58	37	24	15	6	6
228	99	90	73	60	62	42	33	26
229	99	79	59	35	25	19	12	7
230	100	83	65	60	45	34	33	24
231	99	77	54	49	31	24	18	12
	99.39	87.61	72.31	61.89	50.44	40.70	33.44	28.17

Leaf Disk Bioassay Results of Tissue Culture-Derived NE 308 Somaclones with Increased Septoria Leaf Spot Resistance

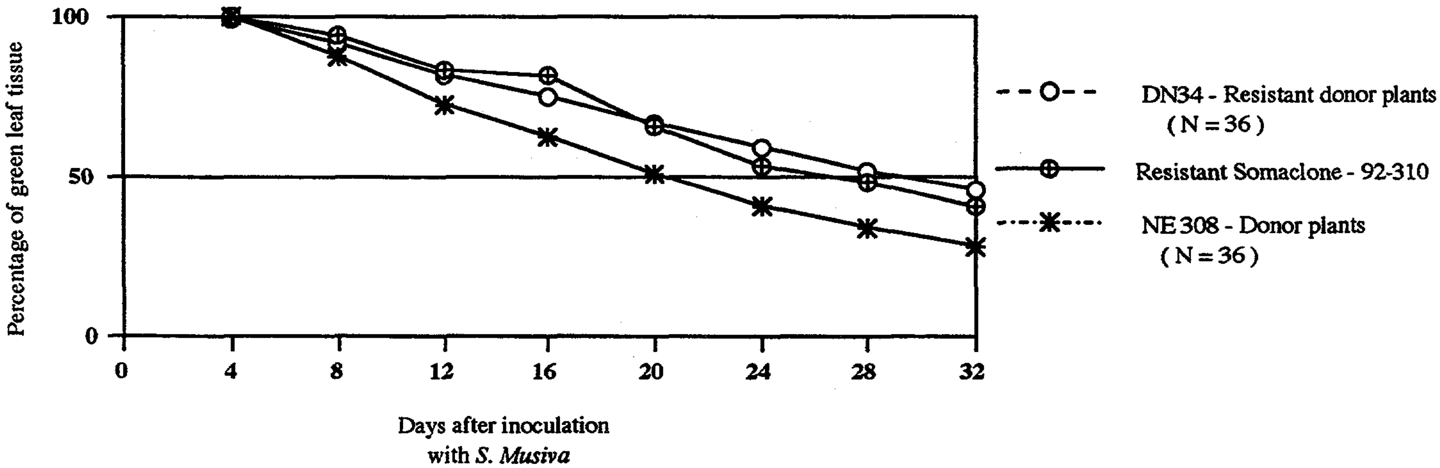
(% green leaf area remaining after inoculation)

Clone	TRIAL #	DAY 4	DAY 8	DAY 12	DAY 16	DAY 20	DAY 24	DAY 28	DAY 32
92-23	231	97	90	82	81	64	52	49	45
	215	99	89	77	71	61	49	49	45
	226	99	88	64	52	29	23	18	12
MEAN		98.33	89.00	74.33	68.00	51.33	41.33	38.67	34.00
92-30	199	100	100	93	88	85	80	75	65
	193	54	31	17	7	3	2	2	1
	194	100	96	82	72	62	54	51	47
MEAN		84.67	75.67	64.00	55.67	50.00	45.33	42.67	37.67
92-45	197	100	100	99	97	90	83	69	67
	194	100	96	94	91	87	88	78	73
	193	99	84	64	60	56	56	52	48
MEAN		99.67	93.33	85.67	82.67	77.67	75.67	66.33	62.67
92-48	215	100	90	80	74	71	61	59	55
	201	100	96	92	90	87	74	68	56
	231	100	93	86	79	53	47	42	35
MEAN		100.00	93.00	86.00	81.00	70.33	60.67	56.33	48.67
92-183	214	100	100	97	94	80	73	64	53
	211	100	99	97	83	82	79	65	47
	229	96	80	67	64	36	32	27	17
MEAN		98.67	93.00	87.00	80.33	66.00	61.33	52.00	39.00
92-191	214	100	99	90	81	90	90	82	78
	226	96	83	64	39	19	10	7	5
	211	100	92	79	75	67	66	62	57
MEAN		98.67	91.33	77.67	65.00	58.67	55.33	50.33	46.67
92-194	214	100	99	92	88	87	85	80	72
	229	100	98	86	71	63	56	50	40
	211	100	100	99	90	82	72	63	55
MEAN		100.00	99.00	92.33	83.00	77.33	71.00	64.33	55.67
92-261	224	100	96	89	89	76	78	65	64
	228	98	97	94	90	89	86	66	58
	**								
MEAN		99.00	96.50	91.50	89.50	82.00	82.00	65.00	61.00
92-310	330	99	87	74	80	51	24	20	3
	222	100	97	79	72	61	57	56	55
	220	100	99	96	92	85	77	67	64
MEAN		99.67	94.33	83.00	81.33	65.67	52.67	47.67	40.67
Overall		97.52	91.79	82.86	76.64	67.53	61.73	54.79	48.16

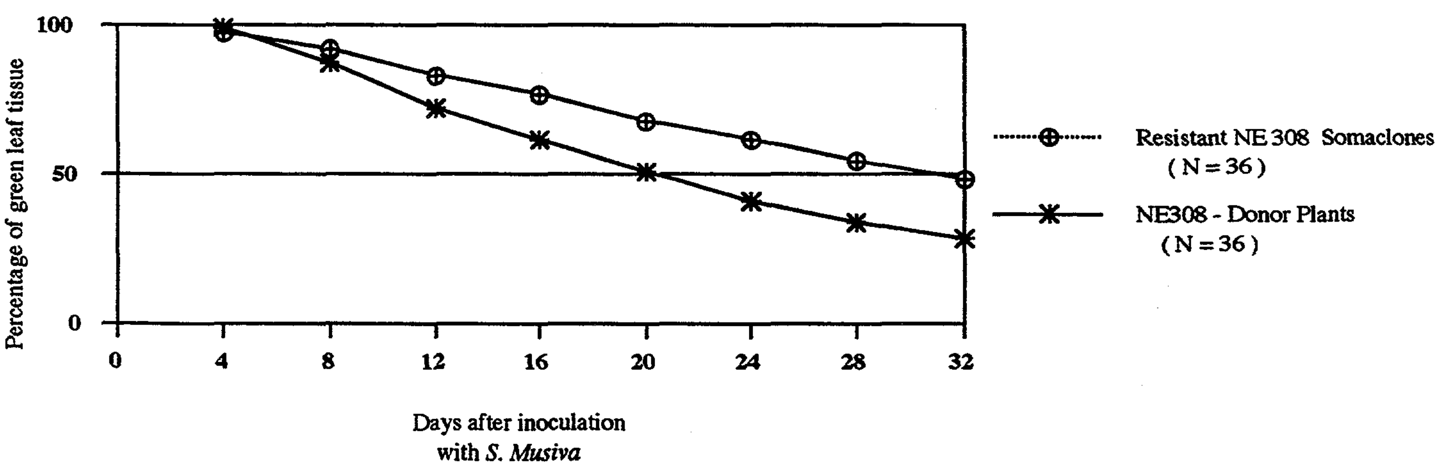
Leaf Disk Bioassay Comparison



Somaclone "92-310" Leaf Disk Results



Grand Mean - Resistant Somaclones



NE 299 Somaclone Planting, Rosemount Minnesota
Ranked by Growth and Disease Resistance

Data Collected Fall, 1994

Clone	Diam. (cm)	Height (cm)	D2H (cm2)	*Septoria* Leaf*	Cankers per Tree	Live Trees	Survival	*Marss.* Leaf*	Winter Injury
Best									
"1-56"	3.3	339	3603	0.9	1.2	12	100%	1	yes
"85-497"	2.8	313	2445	1.0	1.7	11	92%	1	yes
"155"	2.7	326	2407	1.0	1.9	9	90%	1	yes
Good									
"89-6"	2.3	305	1576	1.0	1.5	12	100%	1	yes
"89-2"	2.2	302	1440	1.0	1.7	5	100%	0	yes
"1-1"	2.1	283	1199	1.0	1.7	12	100%	1	no
"85-62"	2.1	260	1126	1.0	1.9	10	83%	0	yes
"85-63"	2.0	269	1059	0.5	0.7	4	50%	2	yes
"87-481"	1.9	274	940	0.8	2.1	9	100%	1	yes
"1-33"	1.9	244	881	1.2	1.1	9	75%	0	yes
"85-573"	1.7	244	719	1.1	1.4	12	100%	2	yes
Moderate									
"85-65"	1.8	252	800	1.2	2.9	12	100%	1	yes
"3-15"	1.7	244	692	0.9	1.3	11	92%	1	yes
"1-7"	1.6	244	643	1.0	1.8	12	100%	1	yes
"89-107"	1.6	227	616	0.8	2.5	11	92%	1	yes
Proto-53	1.6	221	583	1.0	1.3	9	82%	0	yes
"1-10"	1.4	215	426	1.5	2.1	12	100%	0	yes
"89-20"	1.2	221	333	1.0	1.3	10	83%	0	yes
"85-69"	1.3	190	308	1.1	2.0	8	67%	1	yes
Fair									
"89-325"	2.1	241	1049	1.1	4.8	10	91%	1	yes
"89-291"	1.7	234	690	1.3	3.8	4	33%	0	yes
"89-64"	1.6	249	665	1.0	3.2	12	100%	1	yes
NE 299	1.5	247	546	0.8	3.7	11	92%	2	yes
"89-82"	1.5	220	484	1.2	3.4	10	83%	1	yes
"87-63"	1.3	230	395	1.0	3.0	1	100%	2	no
"85-89"	1.4	200	365	1.0	2.5	2	100%	0	yes
Poor									
"1-43"	1.1	230	260	1.2	1.6	10	83%	1	yes
"89-229"	1.1	196	233	0.9	3.4	10	83%	0	yes
"86-69"	1.0	208	226	1.0	0.9	12	100%	1	yes
"89-312"	1.1	186	214	0.9	2.8	7	58%	1	yes
"89-12"	1.1	177	199	1.3	0.8	12	100%	0	yes
"85-507"	1.0	185	189	1.0	0.9	7	58%	1	yes
"85-98"	1.0	195	189	1.6	2.8	7	64%	0	yes
"85-76"	0.9	196	170	1.7	3.3	6	50%	0	yes
"88-106"	1.0	157	142	2.7	0.5	4	100%	0	no
"89-294"	0.5	140	28	1.5	5.0	2	17%	1	yes
Proto-108	0.4	150	24	1.0	1.8	3	25%	1	yes
"87-579"	0.3	198	22	1.3	4.0	3	38%	0	yes
Proto-106	0.4	110	18	2.0	3.0	1	25%	0	yes
"87-130"	0.3	95	9	1.0	0.0	1	100%	0	yes

*0 =absent; 1 =slight, 1 to 25 % crown affected; 2 =moderate, 26 to 75%; 3 = severe,76 to 100%.

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