TOMENTOSUS ROOT DISEASE EFFECTS ON SECOND GROWTH SPRUCE AND PINE IN THE PRINCE GEORGE AND PRINCE RUPERT FOREST REGIONS

by

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Foreword

This Pest Control project was funded by the Canada - British Columbia Forest Resources Development Agreement (FRDA), 1985-1990. Funds were provided unilaterally by the Province under the Intensive Forest Management program, Brushing, Weeding and Pest Control sub-program. Opinions and recommendations in the report are those of the contractor, and not necessarily those of the governments or the FRDA management.

Information and procedures developed herein, although preliminary, should facilitate management of forests with tomentosus root rot in central B.C. Further work is required to provide a firm basis for development operational procedures.

For further information on the report, contact Protection Branch.

J. Muir, RPF
Forest Pathologist
Forest Protection Branch

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I. INTRODUCTION

The following report is a summary of the work completed to date on F.R.D.A. project #25 - Provincial Direct Delivery. The time alloted for project completion is three years. Significant progress in each of the three major study areas has been made during the first year and is outlined below. The studies are progressing as scheduled with the exception of one set back in Study 2 that will be remedied this summer.

An outline of the plans for the 1988 field season has been previously submitted and therefore will not be reiterated in great detail here. To enhance readabililty, much of the fine detail has been omitted but is available upon request. The only results given are preliminary. Final results will be submitted upon completion of each study in the project.

The report is broken down into the three major study areas:

1) Stump excavations
2) Host - pathogen interactions
3) Clonal analysis

Also included is a budget based on the original proposal and the revised plans for the 1988 field season.

II STUMP SURVEY/EXCAVATIONS

The general objectives of this study are to determine the duration of viable _I. tomentosus_ inoculum in old growth stumps, examine the colonization patterns in stumps and the frequency of transmission to surrounding regeneration. More specific subobjectives are given below for each procedure.

This study occupied the majority of the field season and is approximately 3/4 complete. The basic procedures were to identify sites for excavation, do a strip survey on each site to locate colonized stumps, and perform the excavations. Measurements included the amount and location of *I. tomentosus* in stump roots and the number and condition of root contacts with surrounding second growth trees.

Procedures

a) Location of study sites

The purpose of this procedure was to locate sites (clearcuts) for stump excavations and to make some observations on the type of sites on which *I. tomentosus* is most frequently found.

In addition to my own knowledge of areas with *I. tomentosus*, advice regarding potential study sites was sought from several people with the Forest Service in both Prince George and Smithers. Individual cutblocks were located in those areas identified and were examined for evidence of *I. tomentosus*. Criteria for site selection were occurrence of *I. tomentosus* on the site (in amounts necessary for sampling), the presence of spruce or pine stumps and a variety of ages of regeneration trees. Presence of *I. tomentosus* was determined by examining the adjacent stand for disease centres, doing a "walk-through" survey of stumps (partial excavation and examination of stump surfaces) and examination of regeneration trees if present.

Although this is by no means a thorough study of *I. tomentosus* incidence, it does give the location of some more heavily infested areas and provides some basic observations of the characteristics of sites most likely to harbour *I. tomentosus*.

b) Stump selection

For each study site selected, a strip survey of the stumps was performed. The purpose of this procedure was to find 20 stumps with *I. tomentosus* for excavation and to test several methods for use in operational stump surveys. The strip lines were located from a convenient landing, and a bearing was chosen randomly. The survey was not designed to determine the incidence of *I. tomentosus* (because the survey was terminated once 20 colonized stumps were located and lines were not run in areas unlikely to yield *I. tomentosus*) but could be easily modified for this purpose.

Strip lines were 5-10 m wide, every stump in the line was examined for butt rot (surface decay) and three major roots were partially excavated to 1m from the stump centre. Each root was severed or a wedge cut to half the root diameter was removed with a chain saw. The presence of stain or decay was recorded. Samples of decay were collected for isolation and verification of *I. tomentosus*. Stumps with *I. tomentosus* were marked for excavation.

c) <u>Stump excavation and root decay measurements</u>

This portion of the study was designed to examine the factors involved in transmission of *I. tomentosus* to second growth trees: colonization patterns and inoculum viability in the stumps and root contacts with living trees.

Stumps marked for excavation on each site were completely excavated with hand tools. Roots that grew down further than 30 cm were not excavated to their ends.

All roots were given a number and mapped to scale on the tally sheet designed specifically for these excavations (a sample was enclosed with the outline for 1988 summer field work). Roots were examined by axe cuts at regular intervals down their length from the stump centre. Samples of decay and stain were removed, labelled by position and extent of decay (stain, mycelium present, empty pits) and taken to the lab where isolations were made onto 3% malt extract agar (MEA). Figure 2 is a copy of a map sheet which has been annotated to explain what was measured and recorded.

Isolation success from stump root samples was initially very poor but improved greatly as problems associated with contamination from soil and old roots were overcome. The addition of 0.5 to 1% benomyl to the medium was found to increase isolation success. Identification of the fungus was verified by using Nobles Key[1] and description comparisons were made with other fungi that could be mistaken for *I. tomentosus*, such as *Phellinus pini*. Representative specimens were collected to confirm visual identification and to verify questionable cases. Experience in identifying the fungus *in vivo* was deemed sufficient for some of the mapping. Samples were also used in an attempt to estimate the viability of the fungus in different stages of decay.

Results

a) Location of study sites

Table 1 lists the areas examined for I. *tomentosus* for potential stump excavation sites. Maps showing the general location of these areas are in Fig. 1.

In general, where soils are very sandy and shallow *I. tomentosus* was rarely found. Moist, but not wet sites were more likely to have infection centres. Older clearcuts were easier to check for colonized stumps because of the more advanced stage of decay.

b) Stump selection

Selection of the method used to survey stumps for infection by *I. tomentosus* resulted from attempts early in the survey to use an increment corer or power auger which was then checked by

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[1]. Nobles, M.K. 1965. Identification of wood-inhabiting hymenomycetes. Can. J. Bot. 43:1097-1139.

partial excavation and chain saw cuts. The increment corer and power auger underestimated the presence and amount of root decay in young (less than approximately 5 years) stumps. The chips from the auger were difficult to interpret particularily in older stumps. Furthermore, the auger is quite heavy (small chain saws are much lighter) and the amount of time saved boring the stumps rather than cutting into the roots was insignificant relative to the increase in accuracy with the saw.

Table 2 provides information on the number of stumps examined for each site to give 20 colonized ones, and the correlation between butt rot and root decay. Butt rot in older stumps (12 or more years) was observed in an average of 85% of the stumps with I. tomentosus root decay compared to 75% observed butt rot in young stumps. Therefore a survey relying on stump surface decay to identify stumps with I. tomentosus would give a more accurate estimate of incidence in older clearcuts.

Stump excavation

Isolation success was greatest with advanced decay, even very old decay (37-40%). Isolation success from stained wood was less (21%). The fungus remains viable (culturable) in stumps for at least 26 years, and many of the samples from these old stumps had apparently robust mycelium.

Final results, conclusions and recommendations will not be available until the stump survey is complete, but a brief summary of preliminary observations and results for each of the sites follows.

Site 21. The stumps on this site were 15 years old, the regeneration trees were 12 years old. A total of 287 m of roots were excavated, decay with visible mycelium occupied 50% of the root lengths and was found in roots as small as 1 cm diameter. Advanced decay was not restricted to the centre of the root and was frequently found at the root surface where bark was missing or just under the root bark.

Twenty young trees surrounding stumps had a total of 26 root contacts with stumps. Fourteen of the living tree roots had superficial mycelium from which *I. tomentosus* was isolated. In two cases, lesions were found on tree roots near the contact and *I. tomentosus* was isolated. In addition, there was a significant number of tree roots that probably contacted stump roots earlier, but the stump root had since rotted away. Presence of superficial mycelium on roots near the root collar of these trees supports the possibility of earlier contacts. With the exception of one tree, symptoms were not apparent and there was no correlation between leader growth and root contacts or superficial mycelium on roots.

Km 162 (site 29). This was a recent clearcut; the stumps were one year old. Distribution of *I. tomentosus* in recent clearcuts provides a reference point for development of *I. tomentosus* over time. There were no trees established and the site was burnt just prior to sampling. Total length of excavated roots was 608 m, and 50% of the length was occupied by columns of stain and only 10 % colonized by decay. The stain was usually confined to the centre of the roots. Stain columns were small in diameter (usually less than 3 cm) but were found extending upwards of 3m along the root. The usual pattern in colonized roots was advanced decay close to the stump grading into stain acropetally and finally to healthy tissue at 3-5 cm of root diameter. It was also more difficult to locate stumps with *I. tomentosus* because of the lower overall incidence and because butt rot was not as evident.

Pinney ck (site 28). The stumps at Pinney Ck. were up to 24 years old and the area was stocked with residual true fir and spruce with some new ingrowth of spruce and fir ranging from 1-3 years old to approximately 18-20 years old. The stump roots were shorter than in the other sites because of decay; a total of 204 m was excavated and 61% of this was occupied by decay with mycelium present. Much of the decay was adjacent to the root surface. Most roots smaller than 4-5 cm had disintegrated; white pocket rot typical of *I. tomentosus* was generally present throughout the intact length of the root. Twenty living trees had a total of 34 root contacts with colonized stumps. Ten roots in contact with stump roots had superficial mycelium on the surface. A mat of mycelium in dry duff was observed a number of times near stumps colonized by *I. tomentosus* and often near the root collar of residual trees which had become infected. Fourteen second growth or residual trees had root stain typical of *I. tomentosus*; the fungus was successfully isolated from 3 stained root samples.

Km 152 (site 28). Data collection not completed.

Some general and preliminary observations are:

(i) There is a progression of colonization from a predominance of narrow stain columns in newly cut stumps to total advanced decay colonization as the stumps age. This is to be expected because a cut tree has lost it's capacity to resist invasion and colonization by fungi and therefore the decay can progress at a faster rate and approach the rate of advance of stain.

(ii) Decay is not confined to the root centre and will extend to the surface or just under the bark.

(ii) The actual volume of inoculum in the ground and the area it occupies is hypothesized to be low when the stumps are first cut. Inoculum increases steadily to approximately 20 years then begins to decline as the fungus dies out and is replaced by brown rot. Evidence of brown rots moving acropetally along the roots from an origin at the stump was observed.

(iv) Regeneration trees are susceptible to infection from stump inoculum; further work is necessary to quantify susceptibility.

The completed project will provide further information on the above and will put together a picture of _I. tomentosus_ infection in second growth trees throughout stand development. Analysis will include multiple regressions with infection or superficial mycelium incidence as dependent variables. Independent variables will include stand age, distance of regeneration, distribution of the fungus in the root system etc. Regressions will be used to compare situations and to identify influential variables. Peripheral information will include observations and data on infection biology, methods for detecting _I. tomentosus_ in areas to be regenerated, and some incidence estimates.

III HOST - PATHOGEN INTERACTION

The main objectives of this study are to identify differences in host response to infection from several isolate origins and to quantify susceptibility by host species and root size. Such information will aid in developing management options.

This portion of the project was thwarted the summer of 1987 by contamination problems in prepared inoculum. However inoculum has been successfully prepared and will be ready for use this year. Prepared inoculum and natural inoculum will be used to inoculate spruce and pine trees. A variety of variables will be tested which include isolate origin, host species and root size.

As a preliminary test to work out methods for studying tree response and pathogen success and also to provide additional data, spruce seedlings (2+0) were inoculated with several fungal isolates. Several types of inoculum were used and combined with wounding and no wounding in a factorial experiment using colonized toothpicks, colonized dowel and colonized sawdust. These seedlings will be harvested beginning mid March 1988 (3.5 months after inoculation) and will be measured for pathogen advance, host phenol production, starch allocation etc.

IV ISOLATE COLLECTION AND CLONAL ANALYSIS

The objectives of this study are to determine the relative importance of spores and root contacts in disease development.

Vegetative isolates were collected from stumps (spacing) and tree roots, cultured on MEA and stored in MEA slants at 5C. Collections were made from 2 locations near Prince George and 2 near Smithers.

<u>Prince George</u>

(i) Averil Lake. A 50 x 50m grid was established and all trees in root disease centres were mapped. Several isolates were taken from each of 6 disease centres. In addition, 10 disease centres at several distances from the grid were sampled and isolates collected. These disease centres were tied in to the grid.

(ii) Jerry Ck. Ten disease centres were located and sampled within a 60 ha area.

<u>Smithers</u>

(i) Knockholt. A grid was established as with Averil Lk above and isolates were collected from within this grid and from 10 outlying disease centres. Disease centres in the grid overlapped and were hard to define.

(ii) Jonas Ck. Sampling of 10 disease centres is incomplete and will be finished this year.

These represent collections from two isolated populations in each of two geographical regions.

In addition, isolates from eastern Canada, the U.S. and Europe have been obtained. All isolates have been recorded and are on a computer filing system. Pairings of vegetative isolates on petri plate cultures have been initiated. Following one transfer from the initial isolation plate to storage slants and working cultures, the fungus tends to change morphology spontaneously and many different interactions have been observed. This phenomenon makes it difficult to interpret vegetative interactions between isolates. Currently different pairing methods and other means of identifying similar genotypes are under way. One preliminary protein electrophoresis gel has been done and the method looks promising for a coarse level of comparison. Single spore isolates were also collected and will be used in subsequent studies.

Finally, many sporophores were collected, hand sectioned and examined under a compound microscope. No evidence of <u>I</u>.

tomentosus var. *circinatus* was found, all sporophores had straight setae.

V CONCLUDING REMARKS

This report is a summary of preliminary findings and future goals. I hope that it provides the desired information. The following people are acknowledged for their input into the past year of work: Russ Cozens, Bob Richards, Mike Blackstock, Wayne Martin, Maureen Schulting and John Muir of the Ministry of Forests, Everett Hansen of O.S.U. and Duncan Morrison of the Canadian Forest Service. The following corporations/institutions have also provided facilities: Northwood of Prince George, the College of New Caledonia in Prince George and the Prince George Regional Hospital.

Table 1. Survey of stands for potential use as sites for stump excavations.

Stand	I.t.	Name and location	Description
		North Fraser	
1	+	Averil Lake, TFL 25	spaced 1982, 60 yrs old
2	-	Freya Lake, TFL 25	clear cut, recent
3	-	Averil lk, Velpar	clear cut, brushy
		Willow-Cale	
4	-	east of Buckhorn Lk	L58-60, Sx & Pl, dry
5	-	Camp 4 rd.	P1320-M, dry
6	+	George Ck	L75, B75, P77-Sx
		Aleza/Pass Lk	
7	+	Aleza FR, 2km	L65, P69-Sx, patchy
8	-	Aleza and Bear FR	L68, P74-Sx, very dry
9	-	Aleza FR past Bear rd	as site 7, sandy
10	-	Pass Lk rd before Torpy	L68, some decay, suspect
		Hixon Ck	
11	-	Pedley Ck Rd	L75, P77-Sx, sandy, dry
12	-	Pennefather Ck turnoff	L58-69, P77-Sx
13	-	Pennefather Ck	L78, P83, sandy, dry
		500 Rd -George and Jerry Ck	
14	-	George Ck - washout	L60-72, suspect decay
15	-	Jerry Ck rd	L74, P81
16	-	Jerry Ck, at bridge	L74, P76-Sx
17	-	George Ck rd	L60-72
18	-	George Ck rd	L78, P82,84
19	+	500/George roads	in adjacent stand too
20	+	500 rd, Tsinta Ck.	L73, P80, Pl & Ba resid
21	+	500 rd after Tsinta	L71, P75, lots of decay
22	-	500 rd near Jerry	L75, P79
23	+	Jerry, 1 km	S(Pl) 320-M, no stumps
24	+	Jerry ck	L72,73, P76
25	+	Lori Lk Rd	L72, S(Ba,Pl) 110-M
26	-	Past Lori	L72
27	-	Lori rd	L74
28	+	Willow-cale km 152	L87, no regen - Pl and Sx
29	+	Willow-cale km 161	L86, no regen - Sx
30	+	Pinney Ck Rd	L62-70, resid/new Ba Sx
		Smithers	
1	+	Jonas Creek	L61, P64 Sx, pine nats
2	+	Knockholt Burn	Sx 320-M, no stumps
3	+	South of Hwy 16	recent clearcut

L = logged, P = planted

Table 2. Stump survey for *I. tomentosus* in three individual spruce stands.

Stand	No. stumps excavated	A[1]	B[2]	C[3]
site 21	33	18(90)	0	2(10)
Pinney Ck	39	16(80)	2	4(20)
Km 162	55	15(75)	5	5(25)

1. A = number(percent) of stumps with butt rot observed on the stump surface that also contained *I. tomentosus* in the roots.
2. B = number of stumps with observed butt rot but no *I. tomentosus* decay in the stump roots.
3. C = number(percent) of stumps without observed butt rot with root decay.

Fig. 2. Stump excavation Data Sheet.

Site # 21 Transect # 3 Stump # 13 Stump Diam. 50x50

Tree #	Height	Age	Leader (cm)	Distance	Brg.	Symptoms	Contacts
13-1	320	11	31	0.40 m	N43E	OK	R7 = 90, sm
13-2	212	10	30	2.60	N82E	OK	
13-3	248	13	37	1.80	S30E	Stunted [illegible], chlorotic, lesion ok - roots ok	contacts with stump
13-4	190	10	22	3.20	S08E	NONE	roots; Root number,
13-5	260	13	28	3.60	S31W		distance from stump +
							condition of tree

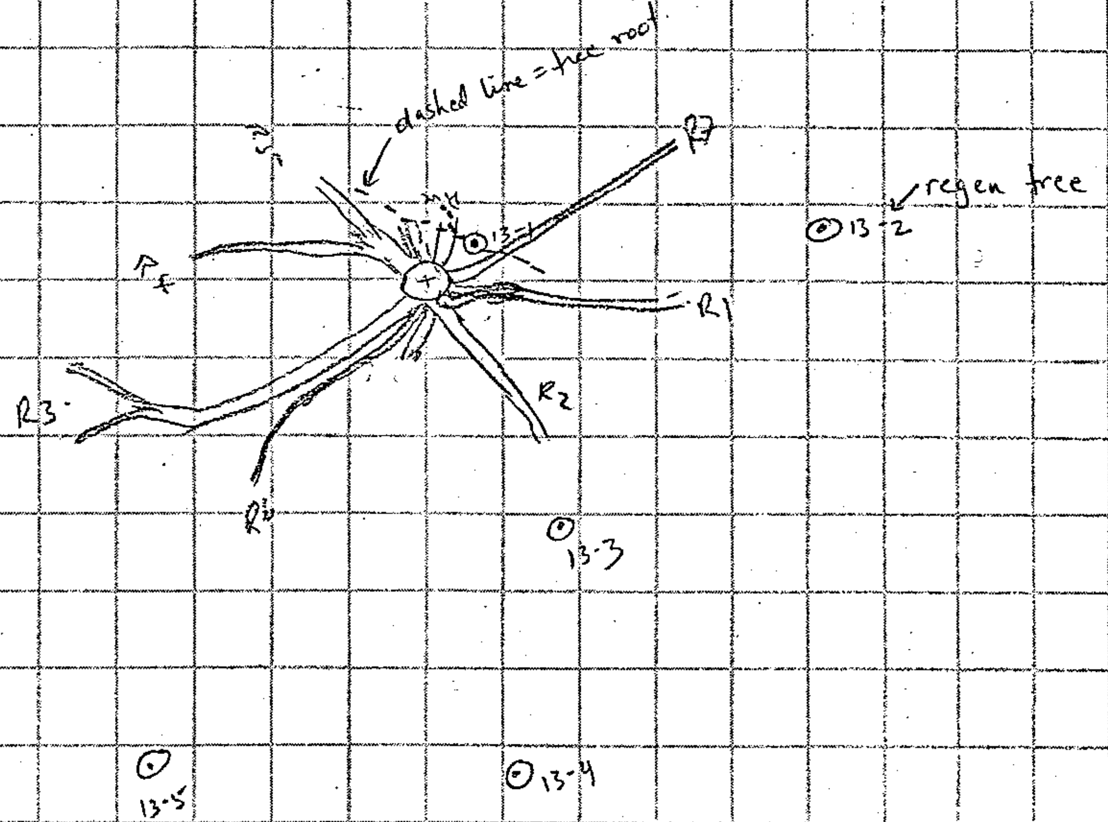
N

5 closest trees to stump.

distance from stump

root - sm = superficial mycelium
L = lesion
H = healthy

1:50 scale



comments:

T13-1 sampled, dug up, yellow sm on root collar extending up 3 cm
sm on several roots - L to cambium - sm quite extensive - to 12cm from root collar

tree 11

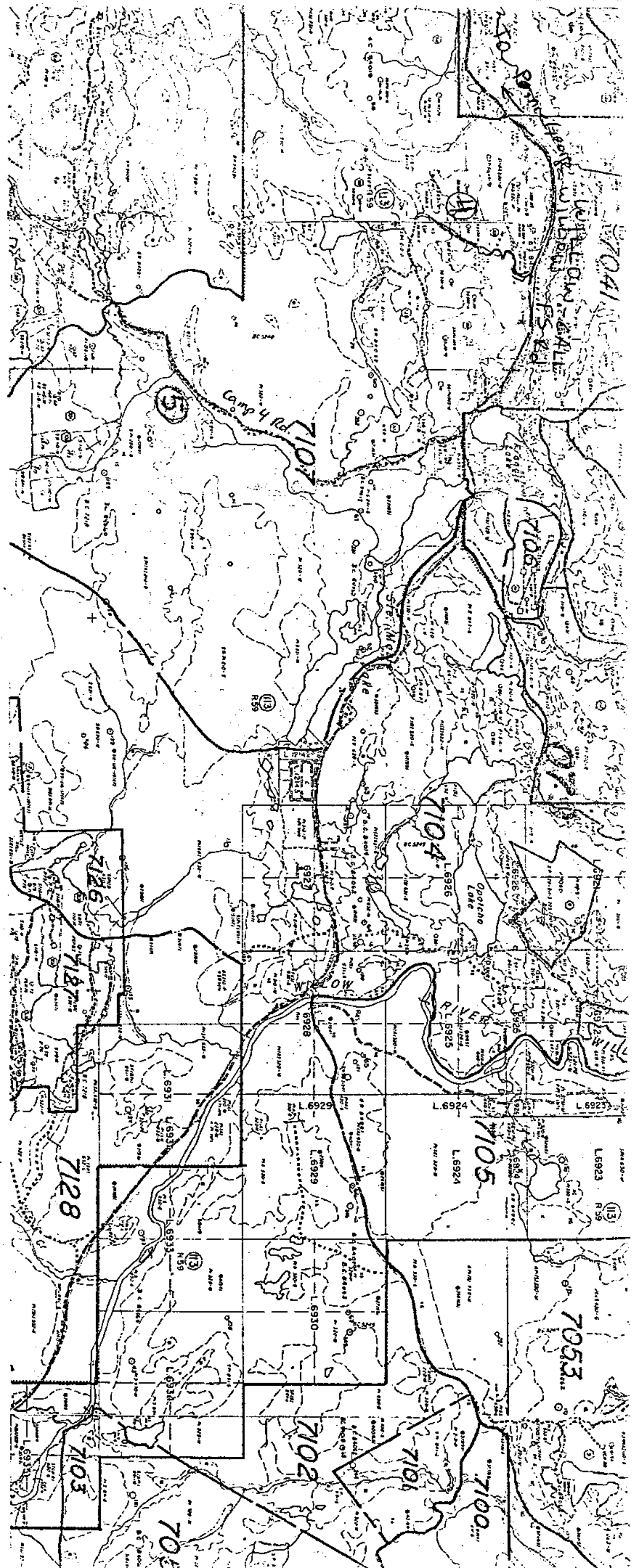
ROOT #	LENGTH RECOVERED (cm)	LENGTH ALONG ROOT (DIAMETER)			CM FROM EDGE	SAMPLES
		EMPTY POCKETS (Ø)	MYCELIUM (Ø)	STAIN (Ø)		
R1	170	BROWN ROT				isolation success.
R2	130	BROWN ROT			60=3 260=0	R3-1 = 60 R3-2 = 260
R3	260		60 - 260 (3)			
R4	150	120 - 150 (2.6)	80 - 120 (3.4)	(0-80 = Br. Rot)	120=0 160=0	R4-1 = 90
R5	120		85 - 120 (7)		120 = 0.2	Root 3, sample 1 at 60 cm.
R6	180	BROWN ROT				
R7	180 / 1190	110 - 150 (7.4)	(0-110 - BROWN ROT)		150=0	
		empty decay pits, or very old mycelium	supposedly active mycelium.		cm from the edge of the root in cross section = how close to the stump root surface is the fungus.	
		distance along root (diam. of root at end of that colonization stage)				

COMMENTS:

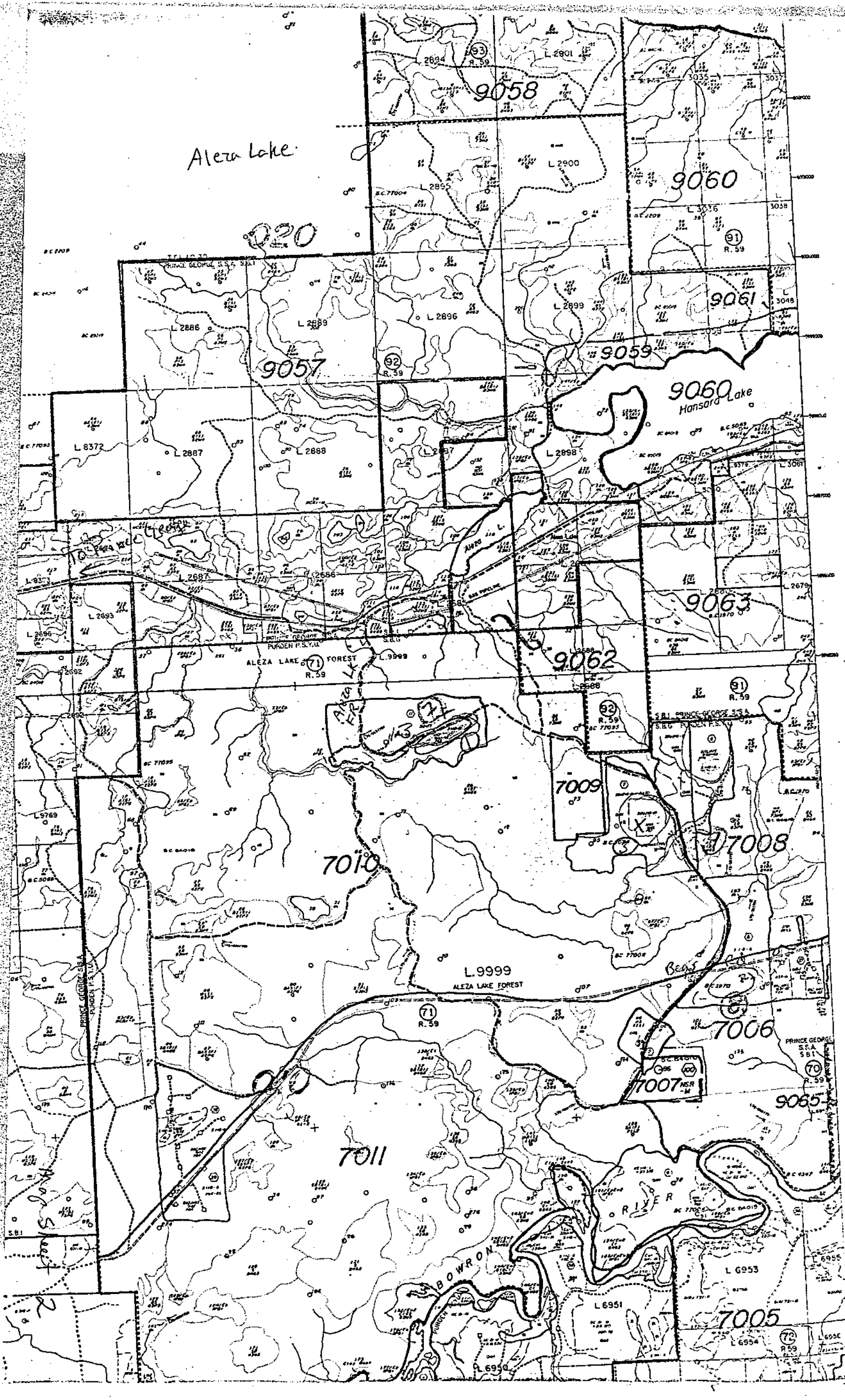
R1 - may have had t. fungus

Fig. 1
Map Sheets
Stump Survey

Ste-Marie/Francis Lakes



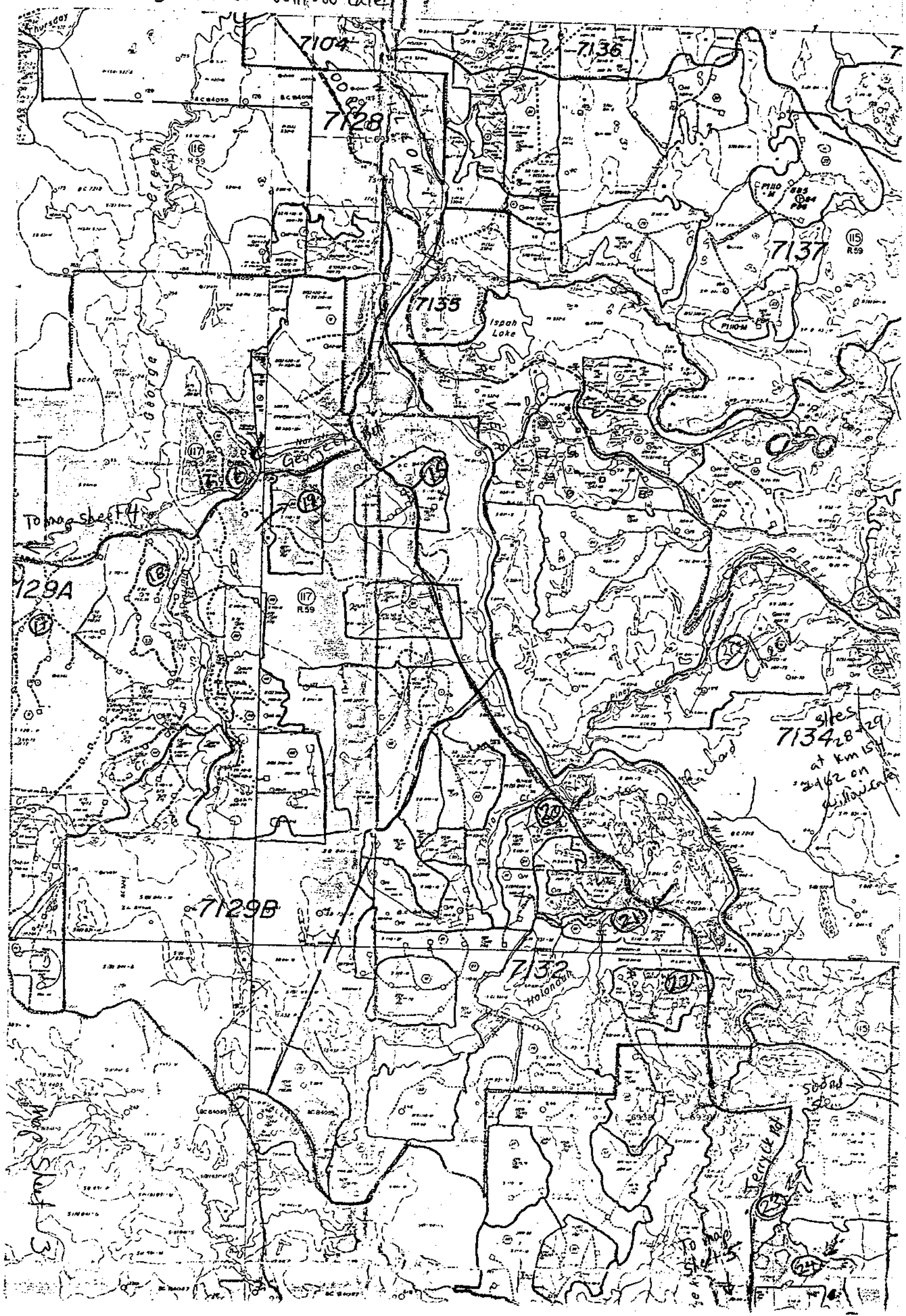
Mg Sheet 1



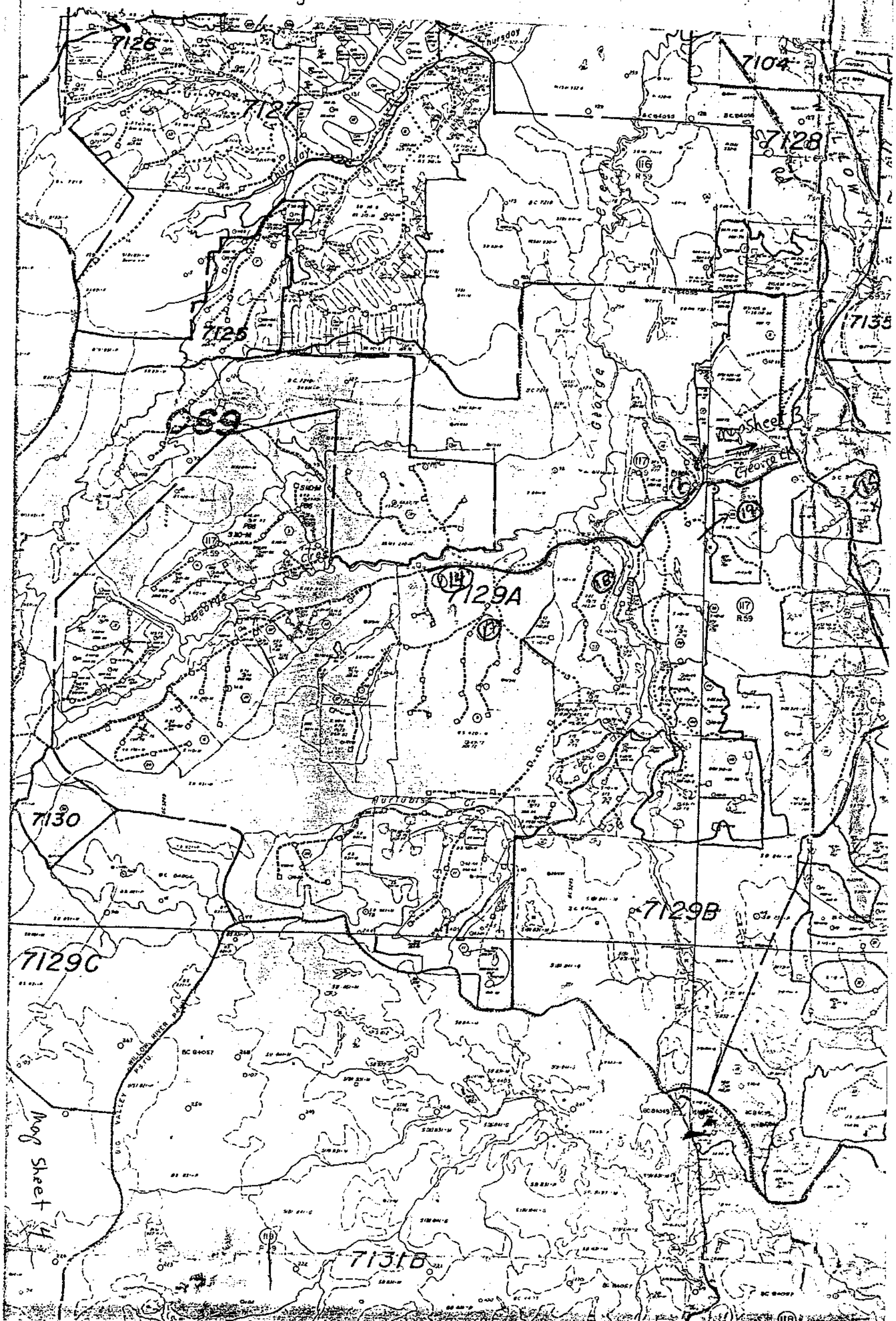
Aleza Lake
020
9058
9060
9061
9059
9057
9060
Hansard Lake
9063
9062
7009
7008
7010
7006
7007
9065
7011
7005
L 2901
L 2900
L 2895
L 2899
L 2896
L 2889
L 2886
L 8372
L 2887
L 2888
L 2898
L 2687
L 2686
L 2693
L 2692
L 9769
L.9999
ALEZA LAKE FOREST
R. 59
PRINCE GEORGE S.S.A.
PURDEN P.S.Y.U.
BOWRON
RIVER
L 6953
L 6951
L 6954
L 6950
L 6955
3035
3037
3038
3048
3061
3016

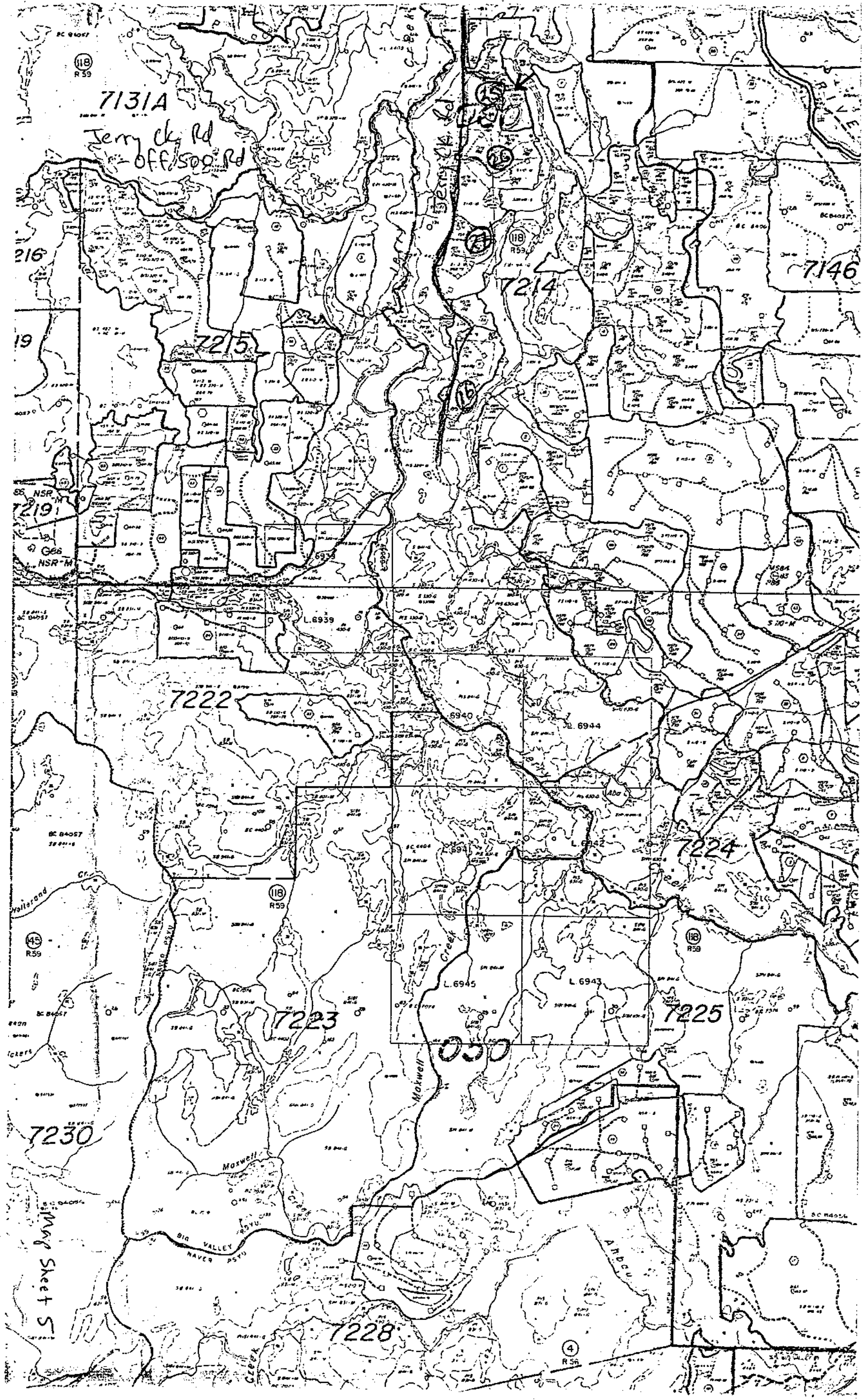
Province of British Columbia | Ministry of Forests | Prince Geo[...] East F.D.

500 Rd off willow Cale

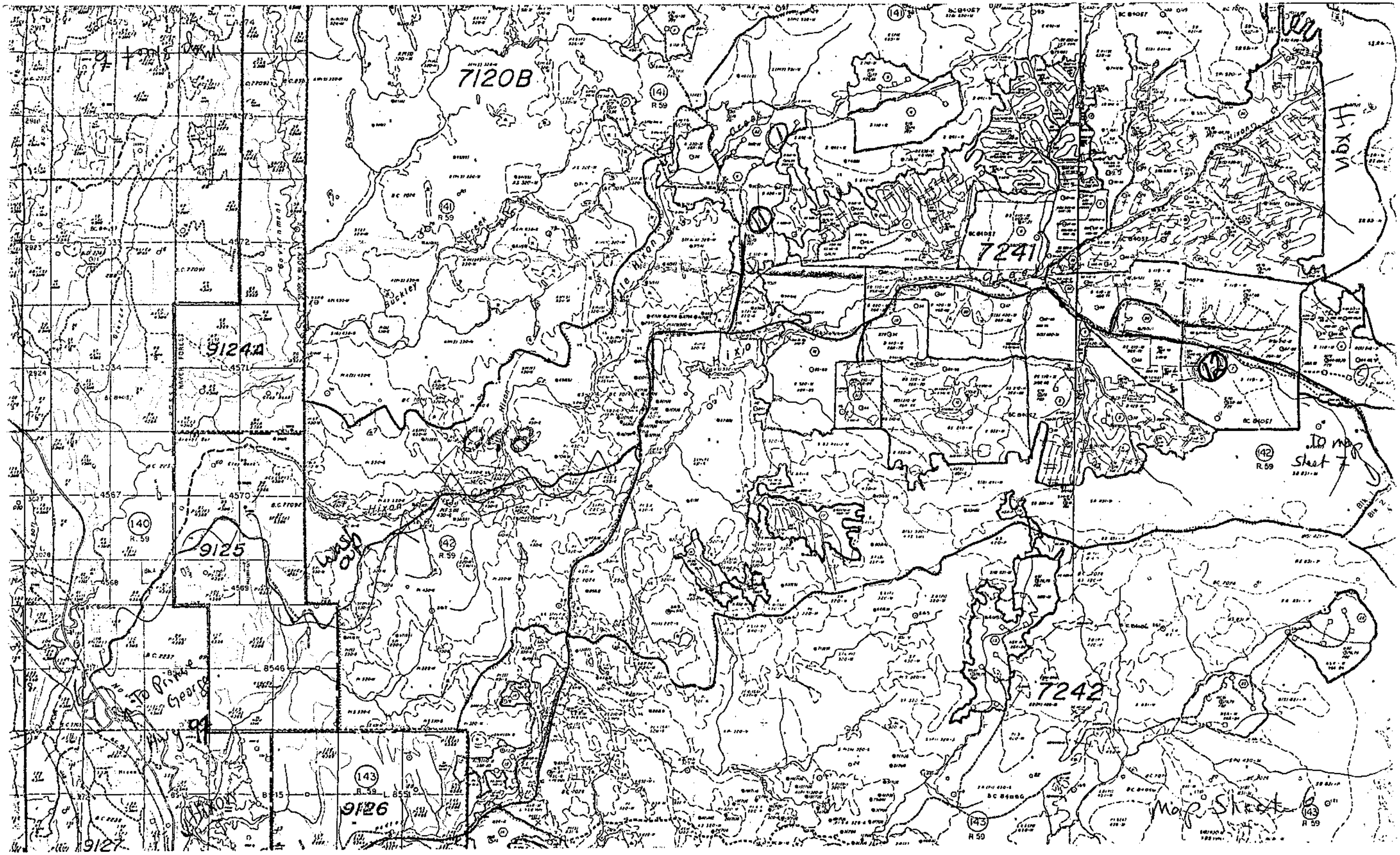


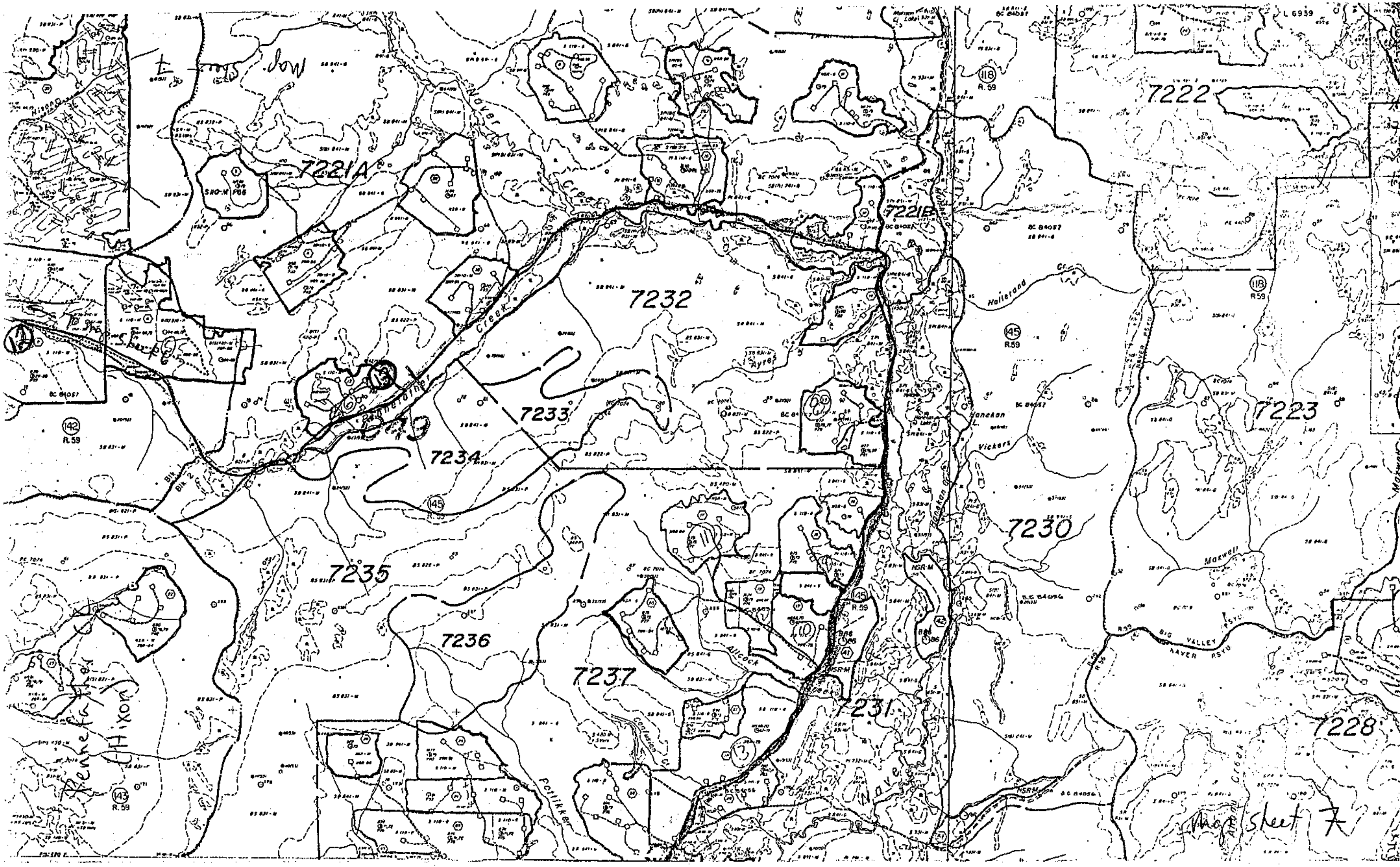
End - George Ck. Rd.





7131A
Jerry ck Rd
off 500 Rd
216
7146
7214
7215
19
7219
NSR-M
L.6939
7222
6940
L.6944
L.6942
7224
L.6945
L.6943
7223
7225
7230
Maxwell
Creek
BIG VALLEY
7228
Map Sheet 5


7120B
7241
7242
9124A
9125
9126
9127
141
R.59
142
140
143
Hixon
Buckley
To map Sheet 7
Map Sheet 6
To Prince George
L.4572
L.4571
L.4570
L.4569
L.4567
L.3034
L.8546
L.8551



7221A
7221B
7222
7223
7228
7230
7231
7232
7233
7234
7235
7236
7237
Naver Creek
Pennefather Creek
Hanekan L.
Vickers
Holleranol Cr.
Maxwell Creek
Allcock
Confusion Cr.
Pettikker
Big Valley
Ayres
Map Sheet 7
Pennefather Ck (Hixon)

Northwood - T.F.L 30