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EFFECTS OF HERBICIDE TREATMENT ON THE INTERNAL PATHOLOGICAL QUALITY OF SURVIVING ASPEN SUCKERS

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ABSTRACT

Nine years after severe damage caused by herbicide (2,4-D; 2,4,5-T) spraying, surviving 14-year-old dominant and codominant aspen *(Populus tremuloides* Michx.) suckers had normal stem stain and decay levels for that age. Widespread top-kill reduced sucker heights, and diameter growth was suppressed markedly for 2 years following the spray. By year 6 normal growth rates had resumed. Extensive stained stemwood that was frequently associated with killed tops apparently spread very little, if at all, in subsequent years, was usually sterile, and never inhabited by potentially harmful, decay-causing microorganisms. Considerable root damage caused by boring insects and *Armillaria mellea (Vahl* ex Fr.) Kummer decay was encountered, but whether or not this is related to the herbicide treatment is uncertain. Some stem stain is generally present in young aspen suckers, apparently because of early branch death, and because of their susceptibility to frequent top-killing caused by many natural agents including shoot blight disease, cold injury, and insects, as well as herbicides. Examination of 160 sprayed aspen trees indicates that aspen which survive herbicide damage have the same potential to produce good-quality crop trees as do unsprayed aspen.

RÉSUMÉ

Les drageons dominants et codominants des peupliers faux-trembles *(Populus tremuloides* Michx.) qui avaient survécu neuf ans après les dommages causés par une pulvérisation d'herbicides (2,4-D et 2,4,5-T) portaient sur leur tige, à l'âge de 14 ans, des traces de coloration et de pourriture habituelles pour cet âge. La mortalité générale des cimes a réduit la hauteur des drageons, et la croissance en diamètre a grandement diminué pendant les deux années qui ont suivi le traitement. À la sixième année, le taux de croissance était redevenu normal. L'abondante coloration du bois de fût, fréquemment associée à la mortalité des cimes, a progressé très peu, sinon pas du tout, au cours des années suivantes; ce bois coloré était habituellement stérile et ne contenait jamais de micro-organismes cariogènes potentiellement nuisibles. On a observé des dommages considérables causés aux racines par des insectes xylophages et par le pourridié-agaric *Armillaria mellea* (Vahl ex Fr.) Kummer, mais on n'est pas certain s'ils sont dus au traitement. En général, il y a coloration anormale de la tige des jeunes drageons de peuplier faux-tremble, apparemment à cause de la mort prématurée des branches et parce qu'ils sont vulnérables aux fréquents dépérissements des cimes causés par un grand nombre d'agents naturels dont la brûlure des pousses, les blessures dues au froid, les insectes et les herbicides. D'après l'examen de 160 spécimens qui ont survécu à la pulvérisation, ceux-ci ont la même capacité de produire des arbres de bonne qualité que les peupliers faux-trembles qui n'ont pas subi de pulvérisation.

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

INTRODUCTION

A common treatment in preparation for planting or release of coniferous trees on cutovers in Ontario is the application of herbicides to kill or retard the growth of competing brush and deciduous trees. In 1978 and 1979, 20,713 and 16,948 ha, respectively, of forest land were sprayed with herbicides in Ontario (Anon. 1979, 1980). On mixedwood cutovers prolific regeneration of trembling aspen *(Populus tremuloides* Michx.), in the form of suckers from the root systems of felled trees, generally occurs in the first growing season after harvesting. Consequently, aspen is frequently the main species affected by subsequent herbicide treatment.

In a broad review of the use of chemical herbicides in the forests of eastern Canada, it has been noted that single aerial sprays generally result in widespread top-kill of trembling aspen suckers but very little mortality, and vigorous resprouting from the roots (Sutton 1958). In Minnesota, aerial spraying with a mixture of 2,4-D and 2,4,5-T completely killed the above-ground portions of 1- and 2-month-old aspen suckers, but new suckers were produced by the root systems the following year (Perala 1971). Two-year-old suckers similarly sprayed had tops "partially killed, causing crooked stems or multiple leaders to develop the following growing season" (ibid.). In another study, aspen suckers that had been sprayed when 5 years old were only partially killed; 5 years later the trees had no multiple leaders, and in most, stem crooks were virtually unnoticeable (ibid.).

Aspen is a common forest component throughout northern Ontario, and its prolific reproductive capacity ensures that it will always be in abundant supply. It is also relatively fast growing. These facts, coupled with the depletion of easily accessible mature stands of conifers, will very likely result in the increased utilization of aspen in Ontario in the future.

The retardation of aspen growth resulting from herbicide spraying is generally of relatively short duration, and the habitual survival and recovery of damaged aspen suckers in sprayed areas will inevitably result in aspen overstories and eventually in trees of merchantable size. The possibility that top-kill and other side effects caused by herbicide spraying may increase the incidence and extent of stain or decay in surviving aspen, thereby lowering the quality of the crop trees, is the subject of investigation in this study.

Many internal stains or discolorations, particularly in deciduous trees, are of physiological origin, that is, they are produced by normal growth or biochemical processes. If they are not invaded by harmful organisms soon after formation, they generally become more or less sealed off and protected from invasion, and the wood remains firm. Other stains or incipient decays contain fungi that can, with time, cause serious advanced, punky decays. There is no simple method, visual or otherwise, of distinguishing between them; therefore, the isolation and identification of microorganisms inhabiting defective wood is important, particularly in predicting future quality of young potential crop trees.

In 1976 the Northern Forest Research Unit (NFRU) of the Ontario Ministry of Natural Resources (OMNR) initiated a study to assess the present and potential impact of a 2,4-D; 2,4,5-T spray treatment carried out 7 years earlier on a (then) 5-year-old aspen sucker stand. Suckers were examined in midsummer in both 1976 and 1978. Staff of the Great Lakes Forest Research Centre (GLFRC) of the Canadian Forestry Service participated in this study by describing internal defects in both stems and root systems of the sampled suckers, isolating and identifying associated microorganisms, determining sucker stem volumes, and in 1976 measuring stem diameter increments. While this is a continuing, long-term study, with a third sampling to be carried out soon, analyses of data collected in 1976 and 1978 by GLFRC are considered to be of sufficient interest to warrant reporting at this time.

MATERIALS AND METHODS

This study was located in the Terrace Bay District on the American Can of Canada Limited timber limits, approximately 45 km east of Longlac and 45 km north of Manitouwadge, Ontario, on the boundary between Site Regions 3E and 3W. Soil within the study area is generally a sandy loam.

In 1965, a mature mixedwood stand was clearcut, and aspen sucker regeneration occurred during the 1965 growing season. The cutover was sprayed on or about 9 July, 1969, in preparation for planting spruce. Fixed-wing aircraft were used for spraying 2,4-D and 2,4,5-T, each at 1.4 kg/ha (active) in a water medium.

In 1976, four 50 m x 50 m blocks were established in the spray-damaged portion of the cutover. Each block was divided into 25 cells, each 10 m x 10 m. Four cells in each block were randomly selected for sampling. The five most dominant aspen stems (potential crop trees) within each selected cell were chosen for sampling, for a total of 80 stems. In 1978, four cells were again randomly selected for sampling within each block, and the sampling method in each cell was repeated. In 1976 a sufficiently large, unsprayed area in the stand to serve as a control could not be found because of the size of the stand and the height of the trees. Fortunately, however, in 1978 OMNR personnel did discover part of the stand that apparently had not been sprayed directly, and a 50 m x 50 m block was established therein. Twenty dominant stems in four cells were sampled according to the same method of selection as was described for the sprayed blocks. Thus, whereas a total of 80 stems were sampled in 1976, 100 were sampled in 1978, including 20 from the unsprayed control block.

The suckers selected for sampling were excavated by severing the lateral roots with a sharp shovel between 25 and 30 cm from the base of the stem. They were transported in this condition to the field laboratory, where total height and height to the base of the herbicide-killed top were measured. Each stem was then cut at ground level and at 15-cm intervals thereafter until the first cut was made in the killed top. From this point on, the former branch that had by then become the leader was cut at 30-cm intervals to within 80 cm of the top. At each cut, the diameter outside and inside bark was measured and recorded on logarithmic tree measurement forms, as were the diameters of any decays or stains present. Cut pieces were further dissected to determine the extent of defects that appeared at one end only. Each decay and stain was described, and, by means of standard cultural procedures and 2% malt agar test-tube slants, attempts were made to isolate microorganisms inhabiting and perhaps causing these defects. The cut faces 15 cm above ground level were kept for subsequent age and increment determinations. A planimeter was used on the tree measurement sheets to determine sucker outside-bark and inside-bark volumes, as well as the volumes of any stain and decay in each stem.

The root systems were washed and brushed clean of soil and debris. The parent roots of each sucker were identified when possible, and all roots were cut 25 cm from the root collar. In this report the term *root collar* refers to the below-ground portion of the sucker adjoining the stem, in which individual roots are indistinguishable. The diameters of the roots at these cuts were recorded except for roots free of stain or decay that were smaller than 12 mm in diameter; these were tallied as small roots. The extent of stain or decay on the cut surfaces was recorded, as were the type and location of externally visible wounds and the presence of internal defect at cuts made adjacent to the root collar. The root collars were cut longitudinally to expose stains and decays which were described and measured. Attempts were made to isolate microorganisms from all stains and decays encountered in the roots and root collars, by aseptically placing small bits of these tissues in test tubes containing 2% malt agar.

STEM GROWTH CHARACTERISTICS

a) Killed Tops

In 1976 it was difficult to find any aspen suckers in the sprayed stand that did not have a prominent dead leader attached, usually in the breast-height region (Fig. 1a, b). By then, 7 years after the spray treatment, all surviving suckers had one branch that had clearly replaced the dead top as the new leader. In most cases this appears to have been a branch that had been

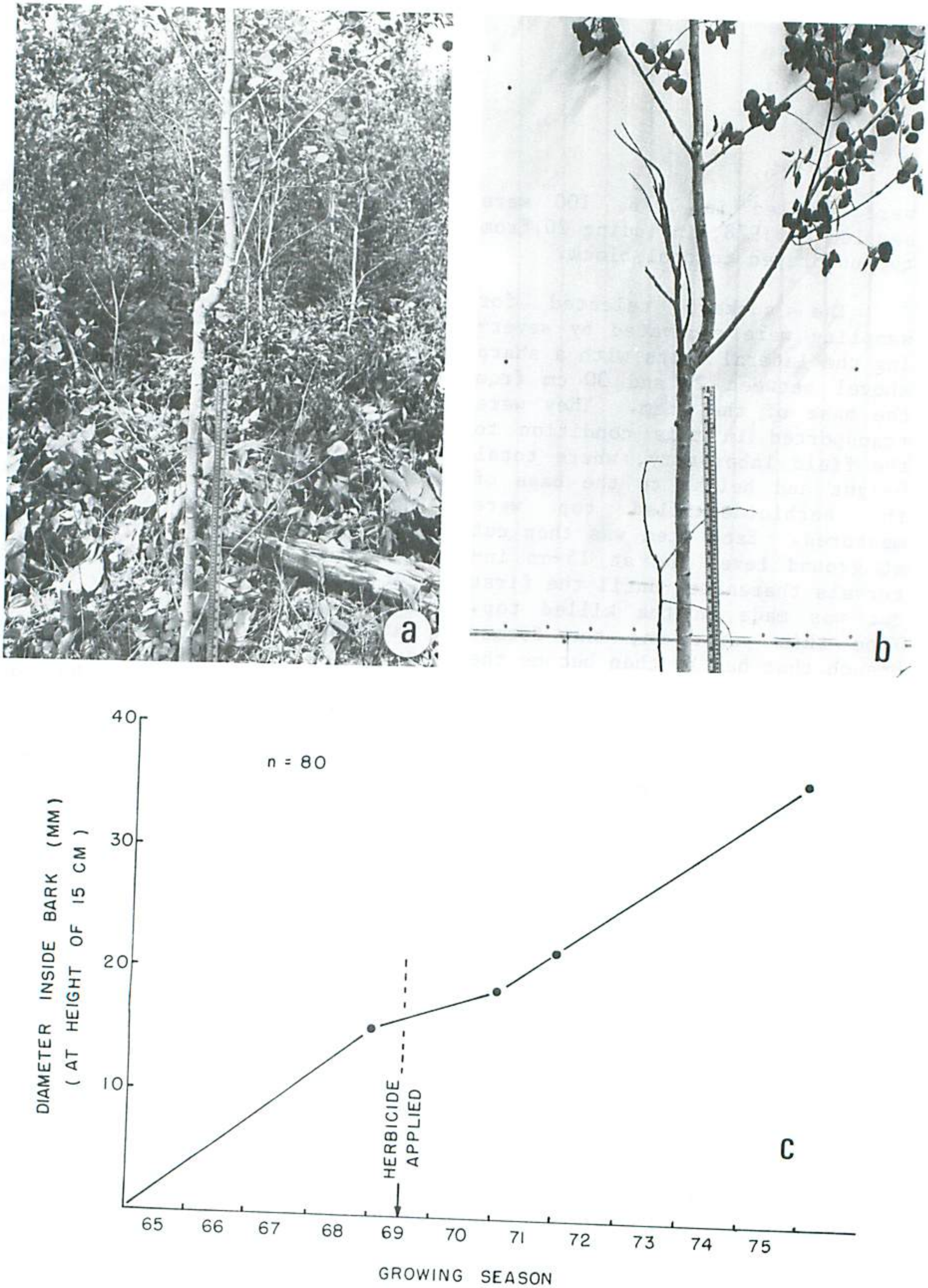


Figure 1. (a) Aspen sucker with herbicide-killed top. Spray applied 1969, photographed 1976. (b) Same aspen as in (a). (c) Apparent effect of 1969 herbicide spray treatment on diameter growth of surviving suckers by 1976.

present when the suckers were sprayed, although in a few instances a dormant bud appeared to have produced the new leader. By 1978 these dead leaders were not as evident, many apparently having broken off in the 2-year period, and the stem crooks at the base of the herbicide-kill were generally less pronounced. More detailed information concerning the effects of the spray damage on sucker height growth will be presented in a forthcoming OMNR report on this study.

b) Diameter Increment

The average annual diameter increment 15 cm above the ground line was measured for each of the 80 sprayed suckers sampled in 1976. All of these suckers had prominent dead leaders in the breast height region which were evidently caused by the 9 July 1969 herbicide spray. In virtually every sucker the 1969 and 1970 growth rings were relatively narrow. In 1971 growth resumed at approximately the pretreatment rate (Fig. 1c). In two older aspen stands (within 20 km of the sprayed stand) that were never sprayed, 1969 and 1970 growth rings were of normal width and showed no narrowing trend (author's unpublished data). This suggests that the narrow 1969 and 1970 rings in the sprayed suckers were caused by the spray treatment and not by climate or other natural influences in the study region.

INTERNAL STEM QUALITY

Wood classified as decayed (discolored wood softer than normal, clear wood) was encountered in 17 (21%) of the 80 spray-damaged suckers sampled in 1976, in 49 (61%) of the 80 such suckers sampled in 1978, and in 12 (60%) of the 20 control suckers sampled in 1978. In all three groups of suckers, approximately half of those with decay had only immeasurable, trace amounts. In the majority of suckers with more than trace amounts of decay, the decay volumes were very small, never more than a few cubic centimetres. Thus, in the 80 damaged suckers sampled in 1976, decay represented only 0.1% of the total inside-bark stem volume. Although the percentage of spray-damaged suckers containing stem decay increased from 21% in 1976 to 61% in 1978, the decay volume in 1978 was still negligible, amounting to 0.2% of the stem volume. The 20 control suckers sampled in 1978 had an average of 0.1% of stem volume decayed.

Every sampled aspen sucker, including the controls, had a measurable volume of stained stem wood (discolored but firm wood) (Fig. 2). The location of the stain in the stem wood of most of the spray-damaged suckers, in relation to the base of the herbicide-killed tops, was similar. As a rule, the portion of the stem just beneath the killed top had the greatest proportion of stained wood. The stain generally extended a very limited distance upwards in the new leader, often disappearing at the point where the

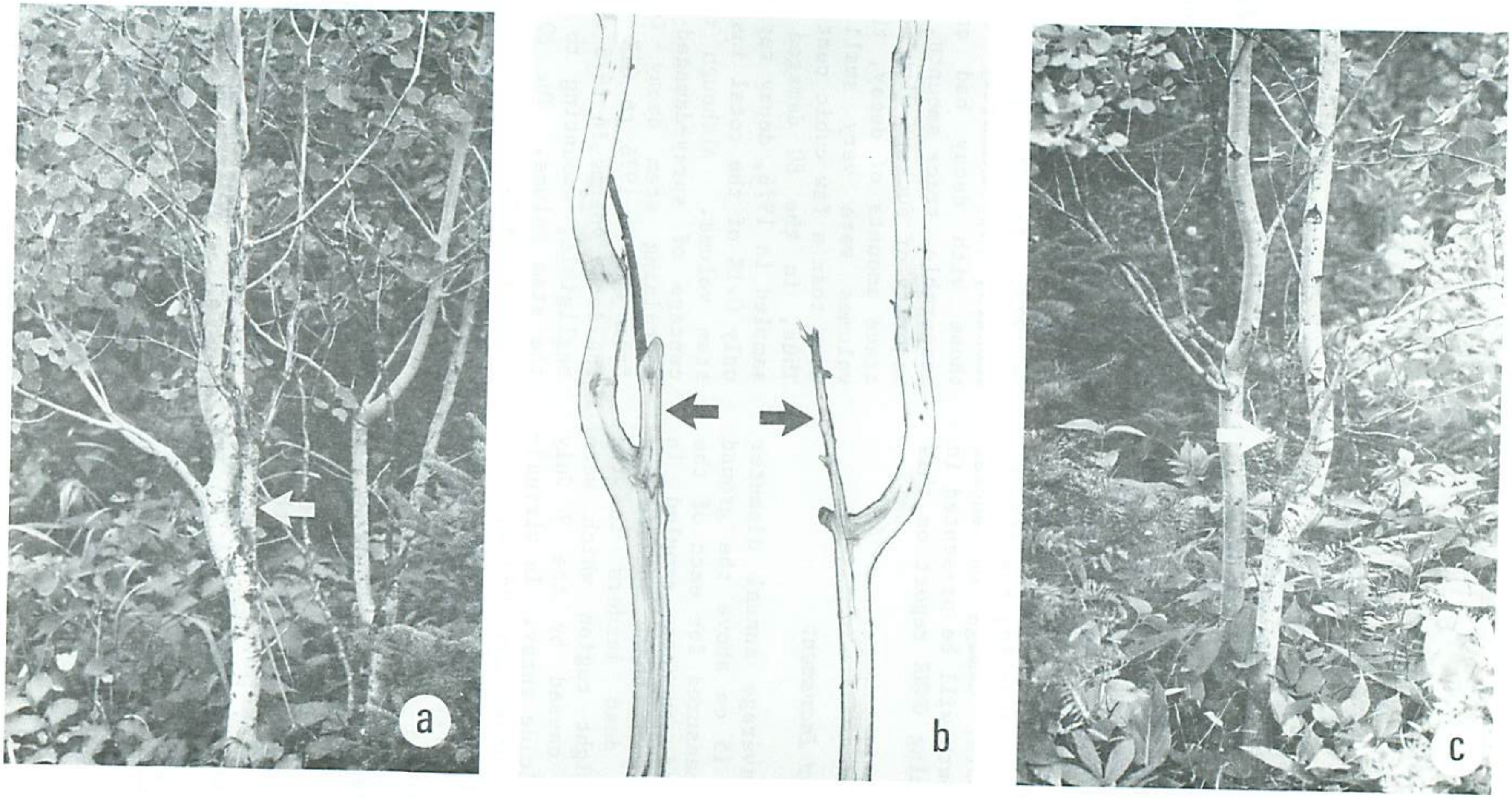


Figure 2. Stained wood associated with killed tops. (a) and (c) Herbicide-killed tops on surviving aspen suckers photographed in 1978, 9 years after spray treatment. (b) Internal appearance of suckers arrowed in (a) and (c), showing stem stain development.

former branch began growing vertically (Fig. 2b). Most of the stained wood extended downward below the killed top, in a very few cases almost reaching ground level, but usually limited to 30 cm or less.

Because of the influence of the position of the killed top on the stain pattern, both 1976 and 1978 samples of 80 damaged suckers were arbitrarily divided into three groups of roughly equal numbers, on the basis of the height at which the base of the killed top occurred. The size of the suckers and the extent and location of stem stain in each such group for the 1976 sample are presented in Table 1 and Figure 3, and for the 1978 sample in Table 2 and Figure 4. It is evident from graphs a, b, and c of Figures 3 and 4 that stem stain was far more extensive below than above the base of the herbicide-killed tops. However, when all 80 suckers are combined (the fourth graph in Figures 3 and 4), this relationship is no longer apparent.

Table 3 shows the average sucker size and stem stain volume for the 1976 spray-damaged trees, the 1978 spray-damaged trees, and the twenty 1978 unsprayed, control trees. The average height of the sprayed suckers sampled in 1978 was slightly more than 1 m higher than those sampled in 1976, and the average DBH was also appreciably greater. However, the average volume of stain per sucker was only marginally greater in 1978 than in 1976 (0.24 dm^3 vs 0.23 dm^3). In the two-year interval between samplings, the average amount of clear, sound wood added to the sucker stems through normal growth was appreciably more than the increase in the amount of stained wood. Hence, the percentage of the total stem volume in the form of stain in spray-damaged suckers decreased from 9.9% in 1976 to 7.6% in 1978.

A comparison of the spray-damaged and control aspen suckers sampled in 1978 is also shown in Figure 5. The average volume of stained wood in the stems of the control suckers (0.33 dm^3) was greater than that encountered in the sprayed suckers (0.24 dm^3). However, the much larger size of the control suckers, presumably attributable to the fact that their diameter and height growth were not inhibited by the spray operation, resulted in a significantly smaller percentage (5.4%) of total stem volume stained (7.6% in sprayed suckers). Figure 5 also reveals that the stain pattern, or location, in the sprayed and unsprayed suckers was quite similar.

CONDITION OF ROOT SYSTEMS

A typical root system of the 12- and 14-year-old aspen suckers examined consisted of a root collar that extended from ground level down about 12 cm, and several lateral roots undulating within the upper 40 cm of the soil. Most lateral roots had several fine sinker roots extending downward. Each sucker stem originated from a root of one of the mature aspens harvested in 1965. The parent root could usually be dis-

Table 1. The occurrence of stain in the stems of 80 dominant 12-year-old aspen suckers 7 years after tops were killed by herbicide spray.

Avg ht to base of herbicide kill (m)	Range of heights to base of herbicide kill (m)	No. of suckers	Avg ht (m)	Avg DBH (cm)	Avg sucker vol, inside bark (dm^3)	Avg vol of stain (dm^3)	Vol stained (%)
1.15	0.85-1.30	27	4.12	3.05	1.70	0.16	9.4
1.40	1.31-1.50	27	4.36	3.43	2.10	0.21	10.0
1.80	1.51-2.10	26	4.79	3.99	3.14	0.33	10.5

Table 2. The occurrence of stain in the stems of 80 dominant 14-year-old aspen suckers 9 years after tops were killed by herbicide spray.

Avg ht to base of herbicide kill (m)	Range of heights to base of herbicide kill (m)	No. of suckers	Avg ht (m)	Avg DBH (cm)	Avg sucker vol, inside bark (dm^3)	Avg vol of stain (dm^3)	Vol stained (%)
0.90	0.50-1.10	28	5.25	3.81	2.99	0.20	6.7
1.20	1.11-1.30	26	5.34	3.66	2.88	0.22	7.6
1.55	1.40-1.80	26	5.64	4.22	3.79	0.32	8.4

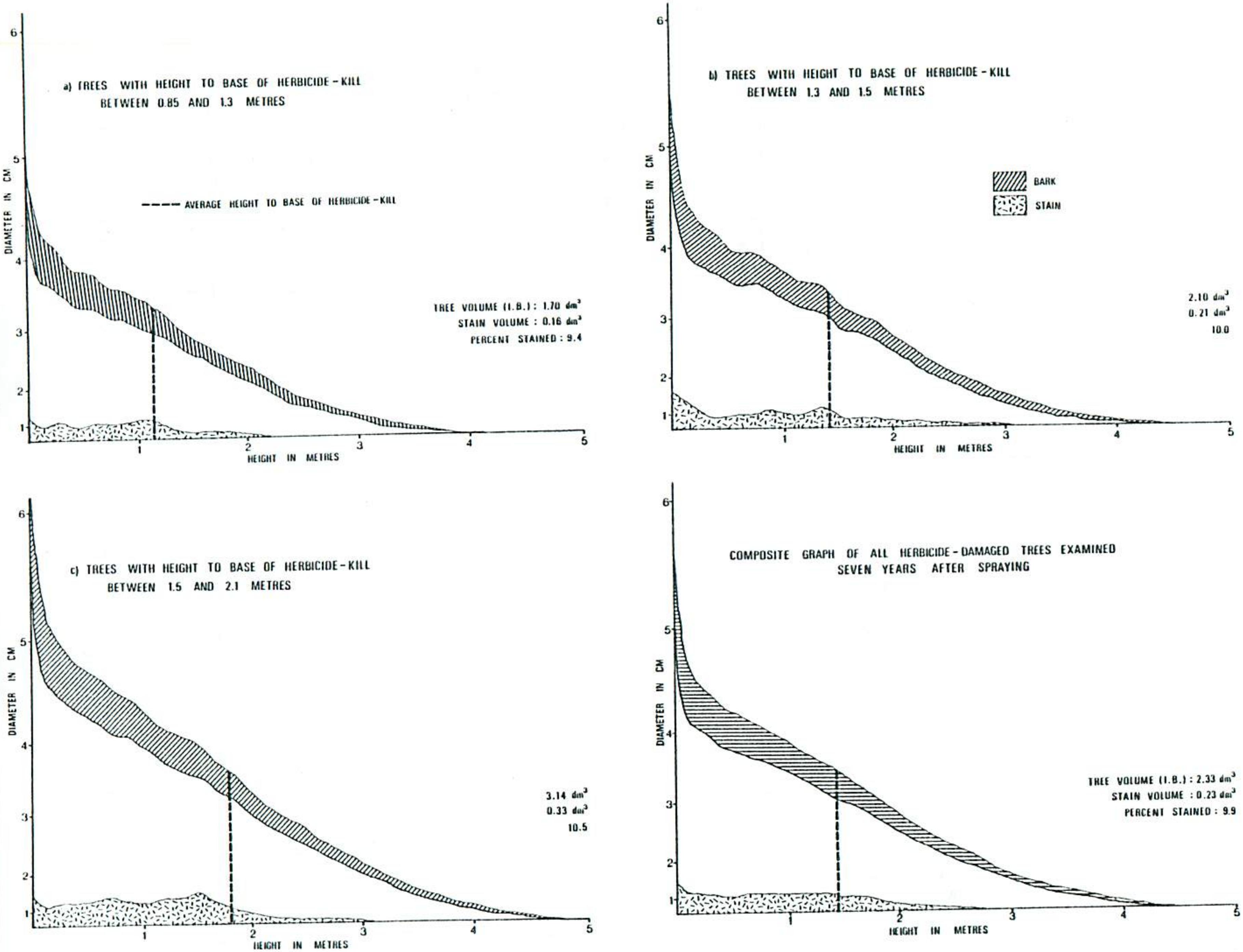


Figure 3. Tree and stain volumes in surviving aspen 7 years after herbicide spray treatment.

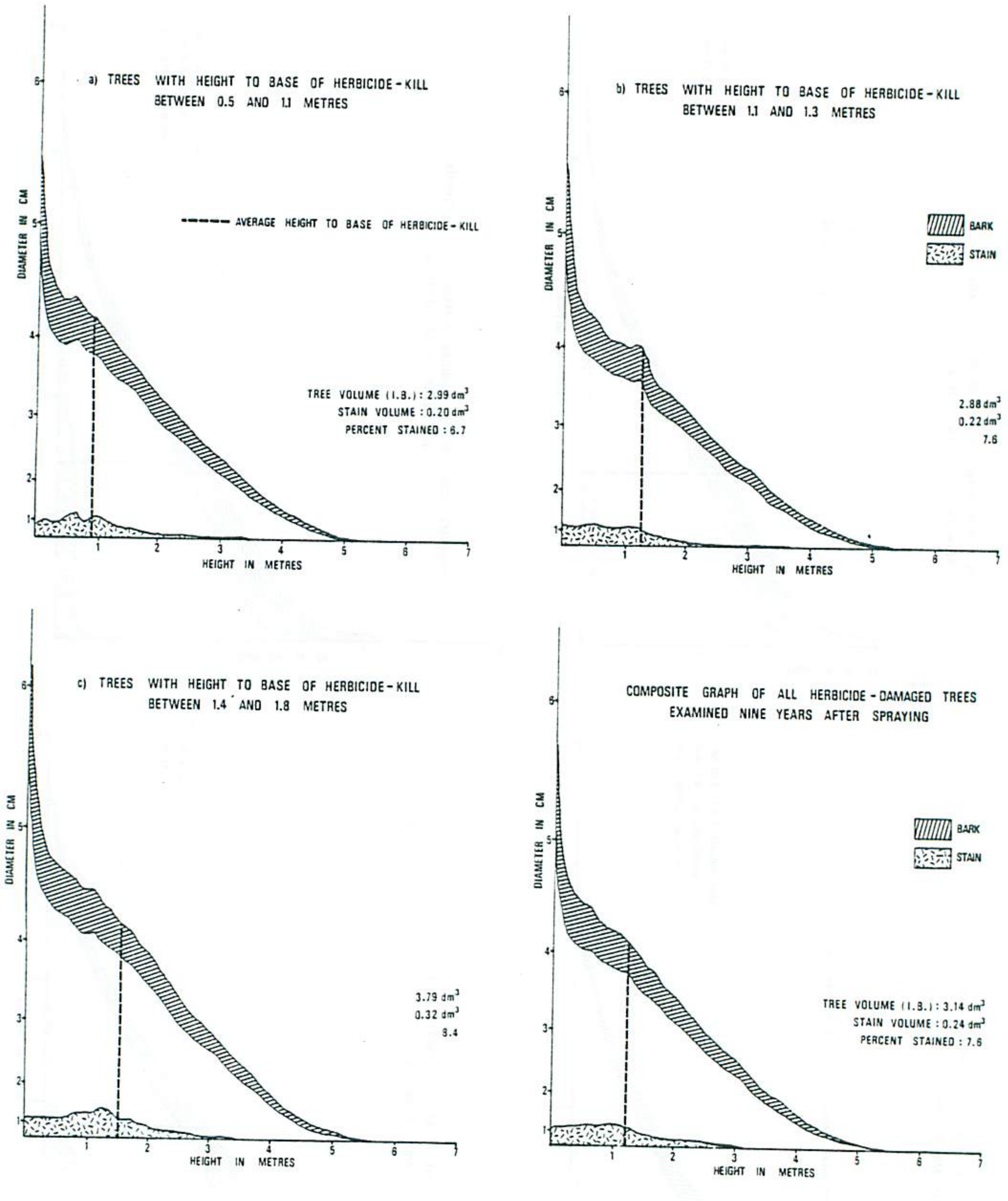


Figure 4. Tree and stain volumes in surviving aspen 9 years after herbicide spray treatment.

Table 3. The occurrence of stain in the stems of dominant aspen suckers with and without herbicide-killed tops. All trees sampled in a single stand with uniform site conditions.

Suckers in sprayed or unsprayed region of the stand	No. of suckers	Years since spray operation	Age of suckers	Avg ht (m)	Avg DBH (cm)	Avg sucker vol, inside bark (dm^3)	Avg vol of stain (dm^3)	Vol stained (%)
Sprayed	80	7	12	4.39	3.53	2.33	0.23	9.9
Sprayed	80	9	14	5.46	3.89	3.14	0.24	7.6
Unsprayed	20	9	14	6.56	4.95	6.11	0.33	5.4

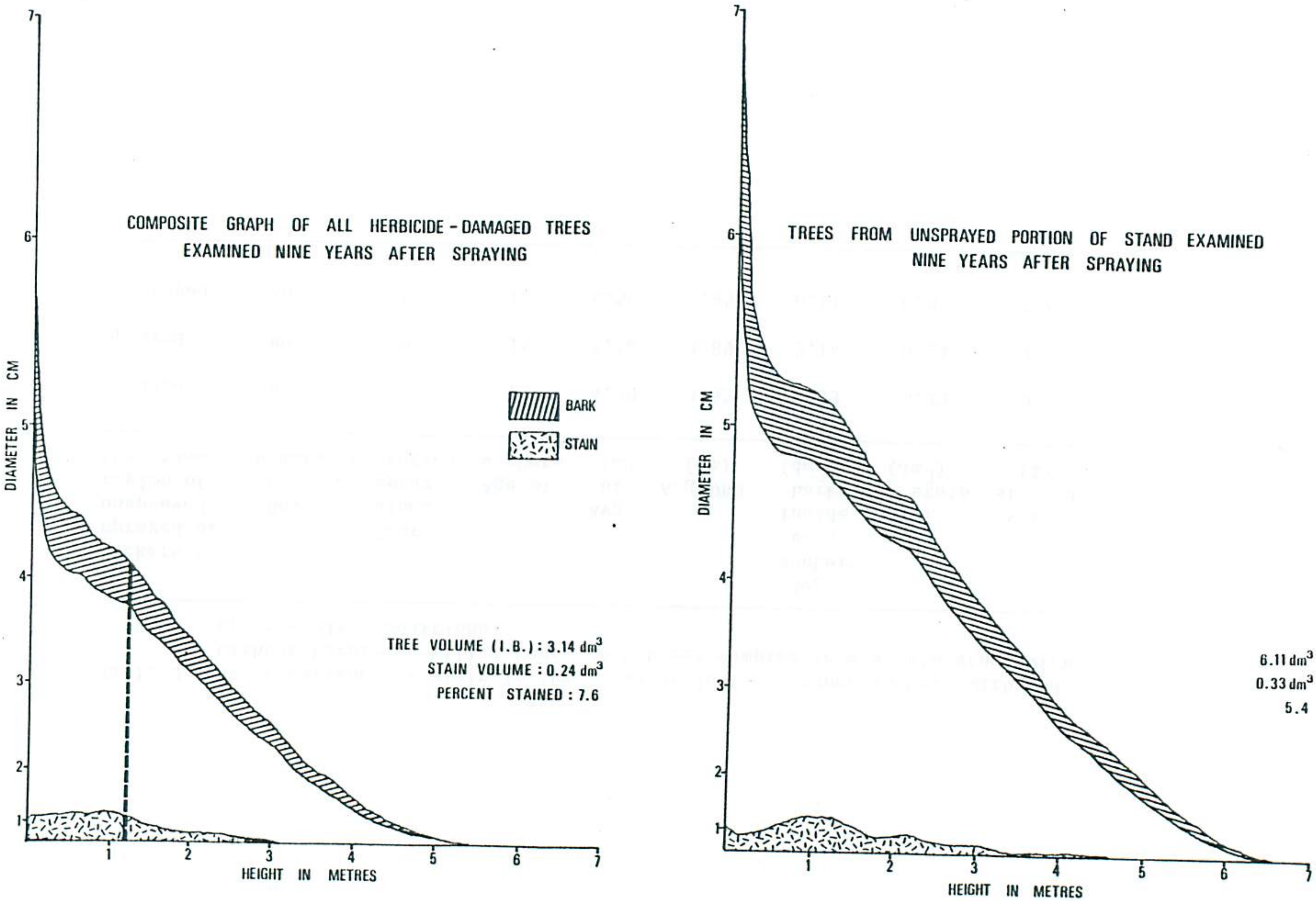


Figure 5. Comparison of tree and stain volumes in aspen suckers from sprayed and unsprayed portions of the stand, 9 years after herbicide spray treatment

tinguished from the new sucker roots by the angles of attachment to the collar-stem axis; these were approximately 90° for parent roots but generally 110° to 125° for the new sucker roots. In relation to the parent stump location the parent root is consistently much larger in diameter on the distal side than on the proximal side (Barnes 1966). Hence, for most suckers a "large parent" and "small parent" root could be identified. An average of 3.5 sucker roots with diameters of 13 mm or more, and 7 sucker roots with diameters of 12 mm or less at the point of severance (25 cm from the root collar), were present for each aspen sucker.

a) Root-boring Insects

The most striking defect in the root systems of the spray-damaged aspen, particularly since it was so unexpected, was the frequent occurrence of large (8-10 mm diameter) tunnels in the roots and root collars caused by larvae of the ghost moth *Sthenopis quadriguttatus* Grote (Fig. 6). Table 4 shows that the root systems of 53.8% of the spray-damaged suckers were tunnelled; in about 15% of those, living larvae were found. The larvae when full grown are about 7.5 cm (3 in.) long and 8 mm (3/8 in.) in diameter (Fig. 7). Tunnels were found in 40% of the unsprayed aspen root systems (Table 4). The larger roots were most commonly invaded, and since the large parent root was usually the largest in diameter, it is not surprising that over half the suckers with tunnelling had tunnels in this particular root. Slightly fewer than half the suckers with larval tunnels had some tunnelling in the root collars, but 11 of the spray-damaged suckers had tunnelling confined to the root collar (Table 4). Cases in which the tunnels extended above ground level into the stem were very rare.

b) Decay and Stain

The root collars of all 20 unsprayed suckers, and of 95% of the 160 sprayed suckers, were defective (Table 5). All of the defective root collars were stained, and 50% of those were decayed as well.

Over one-third (36.2%) of the 890 large lateral roots of the 160 sprayed suckers were defective. Close to half (44.3%) of the 106 large roots of the 20 unsprayed suckers were defective. There were also small, defective lateral roots on the sprayed suckers and two such roots on the unsprayed suckers. The percentage of lateral roots of sprayed suckers that were defective increased from 29.8% in 1976 to 42.7% in 1978. The aspen sucker parent roots had a far higher incidence of defect than the new lateral roots. Among the 160 sprayed suckers, 63.1% of the large parent roots and 61.2% of the small parent roots were defective (Table 5). Comparable figures for the 20 unsprayed suckers were 70% and 75%. On the other hand, only 22.1% of the new lateral roots were defective in the sprayed suckers, and 27.3% in the unsprayed suckers (Table 5).

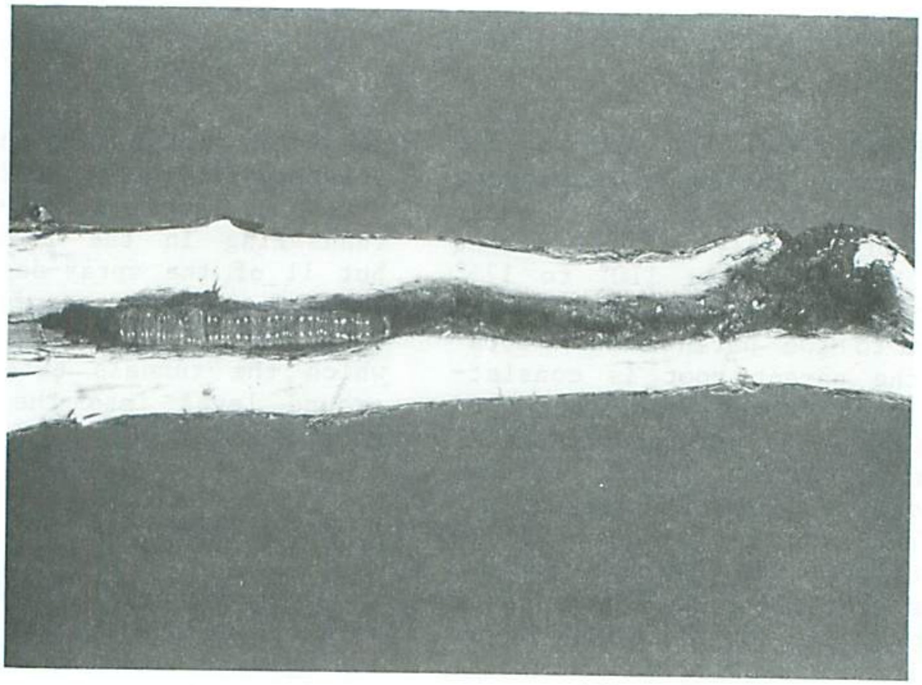


Figure 6. *Sthenopis* larva and tunnel in aspen sucker root, showing ventilation hole at right. (Photograph 1/2 life-size)

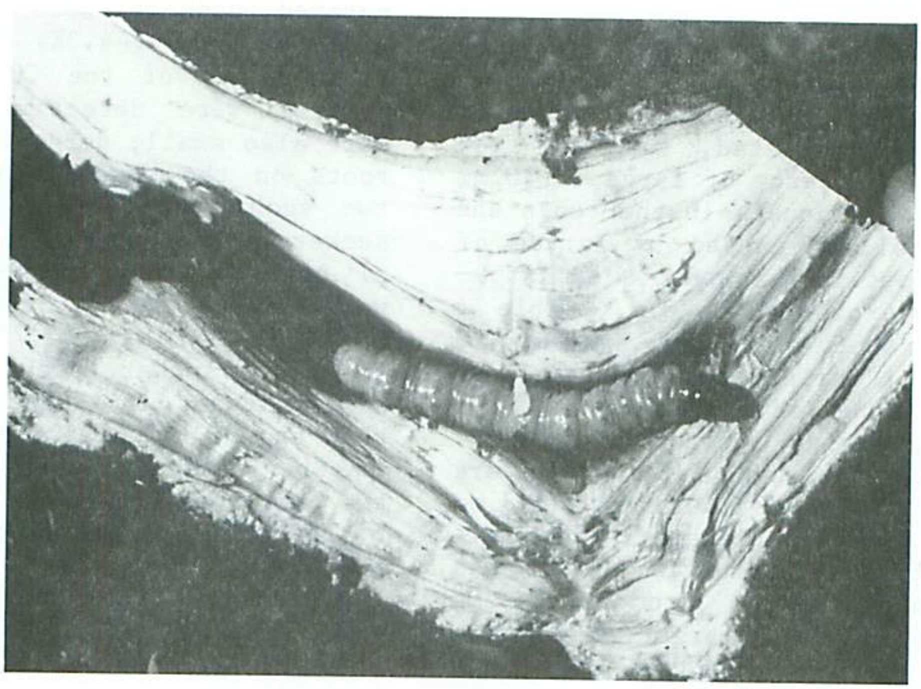


Figure 7. *Sthenopis* larva and tunnel in root collar and large parent root of a dominant aspen that survived herbicide spray damage 9 years earlier (Photograph 2/3 life-size)

Table 4. The occurrence of *Sthenopis* larval tunnels in the root systems of herbicide-sprayed and unsprayed dominant or codominant aspen suckers.

Suckers sprayed or unsprayed	Block no.[a]	Year sampled	Numbers of suckers with larval tunneling: In one or more roots	In large parent root	In root collar region	Limited to root collars	In entire root system
Sprayed	1	1976	8	7	4	0	8
"	2	"	7	3	4	1	8
"	3	"	9	5	6	3	12
"	4	"	11	7	6	3	14
Sprayed	1	1978	14	8	5	1	15
"	2	"	7	3	3	1	8
"	3	"	8	8	6	2	10
"	4	"	11	5	6	0	11
Sprayed	All		75	46	40	11	86
(Percent of sprayed suckers)			46.9	28.8	25.0	6.9	53.8
Unsprayed	5	1978	8	4	3	0	8
(Percent of unsprayed suckers)			40.0	20.0	15.0	0.0	40.0

[a] 20 suckers sampled in each block

Table 5. The occurrence of defect (stain and decay) in the root systems of herbicide-sprayed and unsprayed dominant or co-dominant aspen suckers.

Suckers sprayed or unsprayed	Block no., no. of trees	Defect present in root collars	Number of aspen roots: Parent roots, Distal (large): Total no.	Distal (large): No. defective	Proximal (small): Total no.[a]	Proximal (small): No. defective	New roots: Total no.	New roots: No. defective	All roots: Total no.	All roots: No. defective
Sprayed, 1976 sampling	1, 20	19	20	11	16	10	99	21	135	42
	2, 20	20	20	10	19	9	71	9	110	28
	3, 20	20	20	16	20	10	57	7	97	33
	4, 20	20	20	12	17	8	71	11	108	31
Sprayed, 1978 sampling	1, 20	20	20	16	20	15	109	26	149	57
	2, 20	19	20	13	20	15	61	18	101	46
	3, 20	17	20	10	20	17	35	11	75	38
	4, 20	17	20	13	19	9	76	25	115	47
Sprayed (%):	All,160	152 (95.0)	160	101 (63.1)	151	93 (61.2)	579	128 (22.1)	890	322 (36.2)
Unsprayed (%):	5, 20	20 (100.0)	20	14 (70.0)	20	15 (75.0)	66	18 (27.3)	106	47 (44.3)

[a]In some suckers it was not possible to identify the proximal parent root.

Possible, and externally visible, sources of defect initiation (wounds, etc.) in both root collars and lateral roots were strongly correlated with the occurrence of internal stain and decay. The most common source in root collars appeared to be dead "companion" sucker sprouts, although collar defects were frequently associated with the parent roots or with the basal stem region. Stone abrasions were the most common source of defect in the roots, followed by ghost moth larval "ventilation" holes, dead companion suckers on parent roots, dead root branches, and dead portions of the sample roots.

MICROORGANISMS ASSOCIATED WITH INTERNAL DEFECTS

a) Stems

The occurrence and identity of microorganisms in defective stem wood encountered in the herbicide-sprayed and the unsprayed aspen suckers sampled are presented in Table 6. Since organisms that might enter stems via herbicide-killed tops would most likely progress down the main stems rather than up into the former branches that are now terminal leaders, microorganisms have been separated into those occurring in stem regions below and above the base of the herbicide-killed tops.

Almost two-thirds of the isolation attempts made in defective stem wood yielded no microorganisms in either sprayed or unsprayed stems. Bacteria and yeasts, the most frequently isolated microorganisms, are incapable of causing significant amounts of stain or decay. Basidiomycetes, the group of fungi which includes almost all those that cause decay in the stems of living trees, were rarely isolated. *Peniophora polygonia* (Pers. ex Fr.) Bourd. & Galz. was the only Basidiomycete isolated more than four times (24 isolations), and although it does occur quite frequently in mature aspen it is almost always associated with stain or incipient decay, seldom with soft decay (Basham 1958).

Only 17 of the isolation attempts included in Table 6 were made in the very small pockets of decay found in the aspen sucker stems. Of these, four were sterile, one yielded bacteria, three *Cytospora* sp., two *Ascocoryne* sp., one *P. polygonia*, and six unidentified fungi.

b) Roots

The occurrence of microorganisms in defective wood in the root collars and lateral roots of all aspen suckers sampled in this study is presented in Table 7. Only two fungi, *Armillaria mellea* (Vahl ex Fr.) Kummer (Fig. 8) and *Verticillium* sp., were isolated with any consistency, each occurring 119 times. *Armillaria mellea*, which can cause serious root and butt decay in mature trees, was associated mainly with stone bruises and dead roots. *Verticillium* sp., on the other hand, is not considered a harmful fungus. Roughly 50% of its isolations were apparently associated

Table 6. The occurrence of microorganisms in defective wood of herbicide-sprayed and unsprayed aspen sucker stems.

Microorganisms or sterile	Total no. of isolations obtained from herbicide-sprayed sucker stems	Percentage of isolation attempts: Herbicide-sprayed suckers: At or below base of herbicide-kill		Above base of herbicide-kill		Combined sprayed suckers	Unsprayed suckers
		1976[a]	1978[b]	1976[c]	1978[d]		1978[e]
Sterile (negative)	1173	62.7	60.8	72.5	67.1	63.4	62.6
Bacteria	262	11.5	18.6	11.6	11.9	14.2	12.2
Yeast	78	3.4	4.1	4.3	9.1	4.2	0.7
Cytospora sp.	49	2.9	2.7	1.9	2.1	2.6	3.4
Peniophora polygonia (Pers. ex. Fr.) Bourd. & Galz.	24	2.5	0.3	0.5	0.0	1.3	2.7
Ascocoryne	22	1.6	1.3	0.0	0.0	1.2	0.7
Miscellaneous fungi	38	2.0	2.2	2.4	1.4	2.1	4.8
Unidentified fungi	204	13.4	10.0	6.8	8.4	11.0	12.9

[a]821 isolation attempts were made.
[b]679 isolation attempts were made.
[c]207 isolation attempts were made.
[d]143 isolation attempts were made.
[e]147 isolation attempts were made.

Table 7. The occurrence of microorganisms, and their association with possible points of entry, in defective wood of root systems of both sprayed and unsprayed aspen suckers.

Microorganism, or sterile	No. of occurrences of microorganisms (or sterile) in defective root or root collar wood, by association with different points of entry[a]							
	Stone abrasions (177)	*Sthenopis* ventilation holes (77) or tunnels	Dead "companion" suckers (72)	Partially or entirely dead roots (49)	Dead adjoining roots (45)	Miscellaneous possible points of entry (30)	No obvious visible point of entry	Total occurrences
Armillaria mellea (Vahl ex Fr.) Kummer	38	8	9	26	12	2	24	119
Verticillium sp.	20	60	4	9	8	2	16	119
Phialophora alba van Beyma	8	3	10		2	1	5	29
Ascocoryne sp.			9			2	4	15
Coprinus micaceus (Bull. ex Fr.) Fr.	3		1				1	5
Peniophora polygonia			3				1	4
Miscellaneous fungi	3	4	2	1	2	2	6	20
Unidentified fungi	23	76	18	7	12	13	36	186
Bacteria	13	40	14	4	7	6	65	149
Sterile (negative)	2		3	1	1		19	26

[a]Microorganism isolation attempts were made from most, but not all, root system defects.

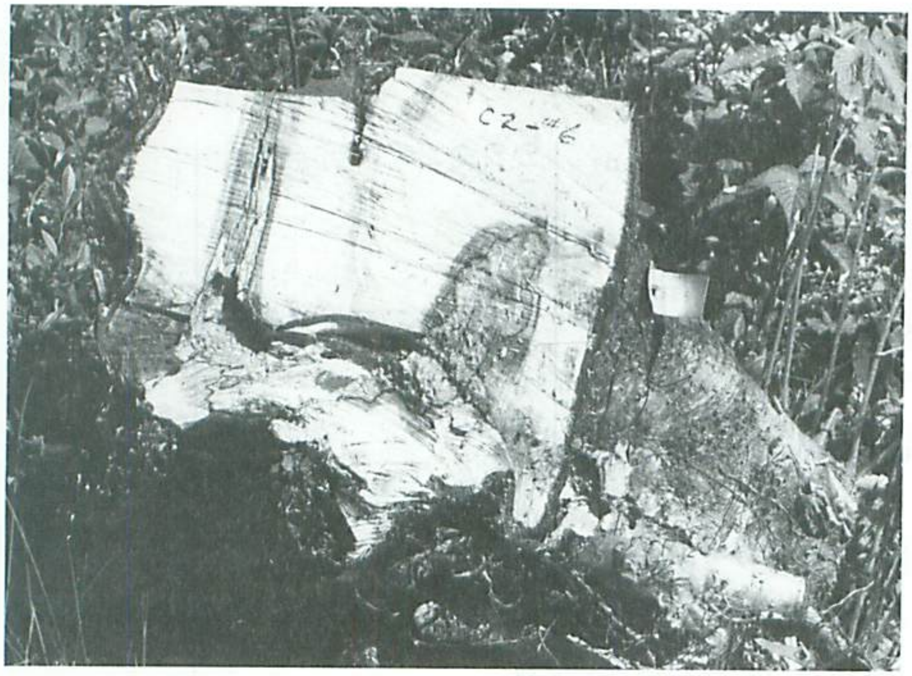
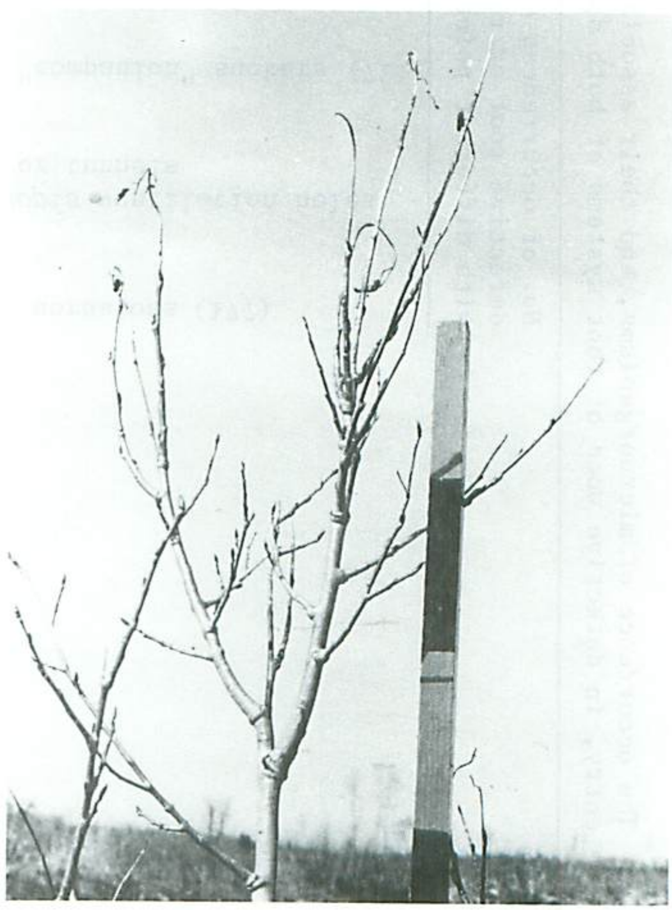


Figure 8

Aspen sucker root collars infected with *Armillaria mellea* root rot. Both stems were sound at stump height of 15 cm.

Figure 9

Aspen sucker with repeated top-kill caused by shoot blight. Each scale section is 1 dm.



with activity of the ghost moth larvae, *Sthenopis quadriguttatus* (Table 7).

Sprayed and unsprayed sucker root systems have not been separated in Table 7, because of the similarity in the frequency of occurrence and identity of the different microorganisms in the two classes of suckers. However, it should be pointed out that *A. mellea* was somewhat more abundant in the root systems of the sprayed suckers. In the 160 sprayed suckers *A. mellea* occurred 108 times (an average of 0.68 per tree) in comparison with 11 times (0.55 per tree) in the 20 unsprayed suckers.

DISCUSSION AND CONCLUSIONS

Within an extensive sucker stand in which almost complete herbicide spray coverage had been attempted, such as the stand in which this study was carried out, it is unlikely that a completely unsprayed, true "control" area exists. There were several features of the sucker stems within the sample block classified as a control that made it obvious that they were not subjected to direct spraying in 1969. These features included the relatively large size of the sucker stems, fewer top-kill symptoms with no concentration in the breast height region, and the comparatively minor diameter growth reductions in 1969 and 1970. The last-mentioned feature indicates that the suckers, though not subjected to sufficient spray concentration to cause top-killing as in the rest of the stand, were nevertheless affected by the spray operation, albeit to a much lesser extent, presumably because of spray drift. Nevertheless, these trees were more suitable than any others to serve as controls because they were subjected to the same climate, site and other envionmental conditions as the sampled, sprayed suckers.

A more detailed account of the effects of herbicide spray on stand density and the growth of surviving suckers will be published by staff of NFRU, who carried out most of the work on these aspects of the study. However, preliminary statements on growth can be made on the basis of observations within the sprayed stand over a 5-year period and diameter increments measured by GLFRC for the 80 sprayed suckers sampled in 1976. These 80 dominant and codominant suckers, which survived the 9 July 1969 spray treatment, had annual growth rings for 1969 and 1970 approximately half the width of those that preceded and followed them. Apparently there was a virtual cessation of diameter growth during 1969 following the spray, since by early July in northern Ontario a little more than half the annual diameter growth of aspen has generally taken place (Rose 1958). The abnormally narrow 1970 growth rings are an indication that the damaged suckers had not fully recovered from the deleterious effects of the spray. However, beginning in the 1971 growing season, ring widths were again comparable with those in nearby unsprayed aspen of similar ages.

The top-killing that resulted from the 1969 spray certainly stopped height growth in 1969; in fact, from the appearance of the suckers sampled in 1976 their average heights by the end of 1969 were probably reduced from what they had been in 1968. Height growth for 1 or 2 years after the spray presumably was below normal, since in most trees several branches probably competed until one assumed dominance and became the new leader. However, by the mid-1970s the new leaders appeared to have overcome any ill effects of the spray and their height growth was normal. The stem crooks resulting from the 1969 top-kill were far less evident in 1978 than they had been in 1976. Nevertheless, despite the resumption of normal diameter and height growth a few years following the spray, Table 3 shows that 9 years after being sprayed the suckers averaged 1 m in height and almost 1 cm in diameter less than the unsprayed "control" suckers.

The frequency with which large, hollow tunnels bored by *Sthenopis quadriguttatus* larvae were found in the root and root collars was unexpected. Though known as a root borer of poplars and willows, this insect was believed, until recently, to be relatively uncommon. The revelation of its potential as a serious root-damaging agent in aspen early in this study was responsible for its inclusion in an Ontario-wide survey of aspen sucker diseases carried out by the Forest Insect and Disease Survey of GLFRC (Gross and Basham 1981). The sucker stands sampled in that survey, which ranged in age from 2 to 10 years, were younger than the stand used in the present study. Nevertheless, *S. quadriguttatus* tunnels were found in 21 of the 45 stands surveyed, and in all regions of northern Ontario (Gross and Syme 1981). In 1977, "major root damage by a hitherto unnoticed root borer" (Vallee and Beique 1979) was discovered in a Quebec plantation of various poplar species and hybrids when the plantation was thinned five growing seasons after its establishment; the borer was identified as *S. quadriguttatus*. One explanation of how the widespread occurrence of this insect in aspen root systems escaped detection for so long is the outwardly healthy appearance of invaded trees (only dominant and codominant aspen were examined in this study). The difference in frequency of occurrence, 53.8% of sprayed suckers and 40% of unsprayed suckers, is of insufficient statistical significance for us to conclude that herbicide spraying renders aspen suckers more susceptible to root borer invasion. Results to date suggest that borer tunnels are associated with limited zones of stained or decayed wood, and that this wood is inhabited mainly by harmless microorganisms such as *Verticillium* sp., several unidentified Ascomycete fungi, bacteria, and yeasts (Table 7).

All the aspen suckers had some stained wood in the root systems, and 86% of the root systems were decayed. Decay was frequently in the form of large pockets or streaks. *Armillaria* *mellea* and *Coprinus micaceus* (Bull. ex Fr.) Fr., two Basidiomycetes that cause serious root and butt decays in mature aspen, were isolated from root system decays, the former quite frequently (Table 7). *Armillaria mellea* was more common in the root systems of sprayed than of unsprayed suckers. Pronos and Patton (1978) showed that the level of *A. mellea* activity was unusually high in the root systems of various species of oaks subjected to chlorophenoxy acid herbicide spraying. Rishbeth (1976) showed that 2,4,5-T, used to inhibit regrowth from hardwood stumps, enhanced the invasion of *A. mellea* via rhizomorphs already present on roots. During establishment of the sample plots late in the 1975 growing season, an unusually high proportion of dead or dying white spruce that had been planted in 1969 shortly after the spray treatment were observed. Without exception, the dead or dying spruce casually examined at that time had root systems with many *A. mellea* rhizomorphs, and with decay apparently caused by *A. mellea*. Research on the possible association of high levels of *A. mellea* root decay in planted conifers with earlier herbicide spray treatment appears warranted, and is planned by GLFRC.

Defect appeared to enter the root collar region mostly via dead "companion" sprouts or via parent roots traversing the collar, but very seldom from the living, sampled stems. The most frequently observed points of entry for defect in the roots were abrasions that resulted from repeated rubbing of the roots (probably caused by wind movements) along irregular stone surfaces. The probability that stone abrasion damage to tree roots is considerably more widespread than indicated by the literature has recently been suggested (Stone 1977).

There is no evidence that the herbicide spraying was directly related to the high incidence of defect in the root systems of spray-damaged suckers. Examinations of root systems of young aspen suckers (Gross and Basham 1981) and of older suckers and 25- to 35-year-old aspen trees (author's unpublished data) suggest that these levels of defect are normal for unsprayed aspen suckers. Indeed, Table 5 shows that the root systems of the 20 unsprayed suckers were slightly more defective than those of the 160 spray-damaged trees.

Table 3 shows that for the 14-year-old aspen suckers examined in 1978, 9 years after the spray treatment, the 20 unsprayed suckers had twice the average stem volume of the sprayed suckers. The unsprayed suckers had an average of 5.4% of stem volume stained whereas spray-damaged stems had 7.6% stained; however, the average actual volume of stained wood was greater in the unsprayed stems. This and other studies carried out recently on young aspen suckers in northern Ontario have revealed that some stained wood can be found in almost any stem, regardless of age or whether or not they have been damaged by herbicide spray, scarification, or other human activities (Smith 1973, Kemperman et al. 1976). One reason for this appears to be that more tops and branches die from natural causes in young aspen than in other tree species, and these dead tops and branches are by far the most common sources of stain development. Most of the extensive stain columns in aspen stems examined in this study, in both sprayed and unsprayed suckers, could be traced to killed tops. Apparently most aspen stands and trees less than 5 m high are very susceptible to shoot blight caused by *Venturia macularis* (Fr.) Müll. & Arx, which infects and kills the long shoots, particularly the leader (Gross and Basham 1981) (Fig. 9). Evidence is accumulating that few aspen sucker stands reach the resistant height of 5 to 7 m without experiencing a shoot blight outbreak, and that most stands upon attaining that height have sustained several outbreaks. During some outbreaks, such as that which occurred in 1975 in the region in which this study was conducted, it is very difficult to find an aspen sucker less than 5 m high without a killed leader. Other natural causes of dead tops include hail, severe frosts after spring flushing, other types of cold injury, and insects. In the region studied in this report, there are records and observations of widespread aspen sucker leader and branch mortality from the severe late frost in June, 1972 and from shoot blight outbreaks in 1974, 1975, and 1978. Stem crooks indicative of leaders killed in the past were evident on the 20 unsprayed aspen, often three or more on a single tree. Hence, it is not surprising that these "control" trees had appreciable volumes of stem stain.

The results of attempts to isolate microorganisms from the defective stem wood in spray-damaged aspen suckers reveal that roughly half the microorganisms that were isolated were bacteria or yeasts, which are certainly incapable of causing decay and are believed to be indigenous microflora in normal, sound aspen heartwood and sapwood (Knutson 1973). Most of the remaining isolations were of fungi which are incapable of causing decay and, for the most part, do not stain wood but are merely secondary colonizers of previously stained wood. Perhaps the most significant result was that two-thirds of the 1,850 isolation attempts yielded no microorganisms, an indication that the stained wood associated with spray-killed tops is primarily of physiological, not pathological, origin. Apparently this physiologically stained wood can extend several centimetres downward in aspen stems, as 60% of isolation attempts made from stained wood 30 to 50 cm below the killed tops were sterile. Many of the herbicide-killed tops were found to represent 2 years' height growth, in contrast with most other forms of top-kill which are generally limited to the current year's growth. Therefore, the relatively large stem diameter at the junction of killed and living tissue would result, as a rule, in more extensive stem stain development than, for example, dead tops caused by shoot blight or frost. However, these stains extended mainly downward, and it was observed that they spread very slowly, if at all, into wood formed in the years following the spray damage. Consequently, although the percentage of stem volume stained in 1970, the year following herbicide spraying, must have been quite high, by 1976 stain amounted to only 9.9% of the total inside bark volume (Table 3). By 1978 this had been reduced to 7.6%, which is approximately the incidence of stain found elsewhere in unsprayed aspen suckers of this age (Smith 1973, Kemperman et al. 1976). The incidence of stem decay in spray-damaged stems, 0.1% in 12-year-old suckers and 0.2% in 14-year-old trees, can also be regarded as normal, since 23-year-old unsprayed aspen have been reported with an average of 0.5% of their stem volumes decayed (Kemperman et al. 1976). Root system damage by fungi and insects was extensive, as it generally is in young aspen. However, its significance and progress in aspen that survive herbicide treatment will be monitored.

In summary, extensive staining of stem wood probably occurred soon after 5-year-old aspen suckers had their tops killed as a result of 2,4-D; 2,4,5-T aerial spraying in July, 1969. This stain is of physiological origin, mostly sterile, with the few microorganisms identified as bacteria and yeasts indigenous to aspen heartwood, or harmless secondary fungi. Despite initial setbacks in height and diameter growth, surviving suckers had, by 1978, resumed normal growth. Stain resulting from the spray damage spread very slowly into stem wood formed after 1969, and by 1978 stem defect in surviving aspen was considered normal. For these reasons, as well as the gradual disappearance of the crooks caused by spray-killed tops, and the attainment by 1978 of a height sufficient to make the trees moderately resistant to any future outbreaks of shoot blight, it is unlikely that the 1969 spray damage will have any permanent harmful effects as far as potential crop tree quality is concerned. A similar conclusion was reached by Perala (1971) in Minnesota. This view is to be tested against subsequent sampling data.

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