RESEARCH REPORT

THE USE OF SCANNING ELECTRON MICROSCOPY AS A TOOL IN DENDROCHRONOLOGY

RICHARD D. REVEL

Faculty of Environmental Design
University of Calgary

INTRODUCTION

Dendrochronological dating of trees from the limits of climatic ranges of tolerance of a species is very difficult as under these conditions the tree is exceedingly sensitive to slight variations in its environmental setting as well as its own physiological processes. LaMarche and Stockton (1974) note that the best correlations between variation in width of annual rings of trees and meteorological records have generally been obtained near climatically determined forest limits such as arid lower forest borders and the Sub-arctic tree line, where even small departures from the climatic norm may limit growth processes within a tree. Daubenmire (1954) suggests that temperature is a major factor in setting the upper elevational limit of the tree line while the work of Mooney, West and Brayton (1966) and Tranquillini (1964) suggests that the subalpine tree line marks a critical altitude above which annual net of photosynthesis is insufficient for tree growth because of the short warm season and low daily maximum temperatures. Thus if net photosynthesis were equal to respiration then one can expect an absence of annual rings, and small amounts of photosynthetic product above respiratory needs would be reflected very sensitively in the annual ring growth.

LaMarche and Stockton (1974) note that, in the Subarctic, the northern forest limit is determined mainly by the length of the warm season and by daily maximum temperatures, both of which decrease with increasing latitude. Eklund (1957), Giddings (1943), Hutich (1948), and Mikola (1962) suggest that at the northern tree line the temperature of the warmest months is the only significant factor correlated with tree growth.

The sensitivity of annual rings, and consequently the ability to recognize very small increments in annual growth, is further complicated by the fact that the tree crown is the primary transpiring surface and also the principle synthesizer of growth regulators (Kramer and Kozlowski 1960). As the tree ages and elongates, the lower branches die with the result that mean crown position is higher up the stem and materials must travel a greater distance to a given cambial area with the result that competition within the tree occurs for photosynthetic products (Fritts 1966). Further, as Larson (1962) notes, cambial initiation occurs last in the basal cambium while growth cessation often occurs there first thus providing a shorter growing period at the base of the plant.

Because of the importance of being able to study tree growth patterns from trees at or near their ecological limits to the fields of glacial geology, dendroclimatology, archaeology and ecology, and also because of the extreme difficulty in ring discrimination in specimens from these areas due to their ultra sensitivity, it is the

intent of this paper to document a technique using scanning electron microscopy which the author has found very useful in ring analysis of ultra sensitive *Picea glauca* (Moench) Voss.

SPECIMEN COLLECTION

During July 1979 specimens were collected from two sites in the arctic tundra as part of a research investigation into the proposed Anderson River Ecological Site (Revel 1979).

Site #1 was located in the arctic tundra approximately 400 north of Krekovick Landing and 100 east of the shore of Wood Bay on the Arctic Ocean. Here a single Krumholtz *Picea glauca* tree was encountered growing approximately mid slope on a 300 m slope with a southern aspect. The surrounding vegetation was predominantly an open birch-willow shrubland with a dense moss mat dominated by *Tomenthypum nitens.*

The *Picea glauca* (Moench) Voss was severely contorted and grew in a creeping shrub form. The maximum length of the main stem was 1 m and the maximum diameter of the tree trunk was 2.5 cm outside bark and 1.9 cm inside bark at the oldest part. The main stem of the tree grew prostrate to the ground and was covered over by a thick layer of moss with needle bearing branches growing vertically from it.

In order to determine where the main stem of the tree was rooted, it was necessary to clear away the moss by starting at one of the protruding branches and carefully working backwards to the point at which the principal root entered the soil. Once this was located, the tree trunk was cut at the base and the entire tree removed for tree-ring analysis.

Site #2 was located approximately 55 km upstream of Krekovick Landing along the west shore of the Anderson River. Here a single specimen of *Picea glauca* (Moench) Voss was located on a river levee approximately 12 m west of the river bank. The specimen grew in a suppressed tree-like form to a height of 4 m and had a basal diameter at ground level of 6.2 cm. Accompanying vegetation on the levee was predominantly 1-2 m tall *Salix glauca* L. and the site was bounded to the east by the Anderson River and to the west by a low lying wet sedge (*Carex* spp.) meadow.

A tree core was extracted at the base of the tree using a standard Swedish increment borer after removal of the dense lowermost tree branches and some of the moss surrounding the base to provide working space. The core was taken at the point of maximum diameter of the tree and traversed the entire tree diameter rather than the more standard method of boring just to the tree centre. This method was used to ensure that a sample of maximum radius was collected whereby rings might be more readily discernable than on the narrowest radius. The core was preserved by encasing it in masking tape and assigning it a reference number for later identification.

VISUAL EXAMINATION

After returning to the laboratory, both cores were examined under a standard dissecting microscope. Annual rings on the more southerly specimen collected on the river levee were readily discriminated and no difficulty was experienced at fixing an age of 105 years for the tree.

The age of the more northerly specimen however was impossible to determine using the same techniques due to the ultra sensitivity of the annual rings. Before

examination a cross section 5 mm thick of the entre tree stem was cut and the section polished with successively finer grits of sand paper until a smooth surface was formed. By visual inspection rings could be discriminated for the first 40 years of growth but at that point the rings become so close and the early wood — late wood boundaries so ill defined that any attempts at ring analysis would have been nothing short of conjecture. Discussions with L. A. Jozsa of Western Forest Products Laboratory in Vancouver indicated that he had reservations about the ability of x-ray densiometry to discriminate rings; however, a cross section of the tree was left at that laboratory for later experimentation.

It was at this point the author decided to explore the possibilities of using scanning electron microscopy in a final attempt to solve the problem. Consultation with Mr. Lazlo Veto, electron microscopy technician in the Department of Botany, University of British Columbia, confirmed that, although he was unaware of the technique being used in tree ring analysis before, he thought it might work using their Cambridge model 250T scanning electron microscope (SEM).

SPECIMEN PREPARATION AND PHOTOGRAPHY FOR SEM ANALYSIS

Closer examination of the sanded surface of the cross section of the northernmost tree indicated that the cell edges were rough and would probably provide poor photographs under the higher magnification provided by the SEM. To rectify this a fresh disposable scalpel was used to cut a smooth V shaped surface from the cut side of the bark to the centre. The entire cross section was then placed under low vacuum in a warming oven for four hours. It was then removed and glued to a standard SEM metal stubb using a carbon based adhesive and placed back in the same warming oven under low vacuum to dry (Figure 1). In mounting the stubb, care was taken to ensure that the stubb was located beneath the entire radius of the tree to be examined. Examination of the dry carbon-adhesive joint indicated extensive cracks which would have interfered with results in SEM analysis so these were filled in with a silver based adhesive and left once again to dry. The specimen was then placed in the high vacuum

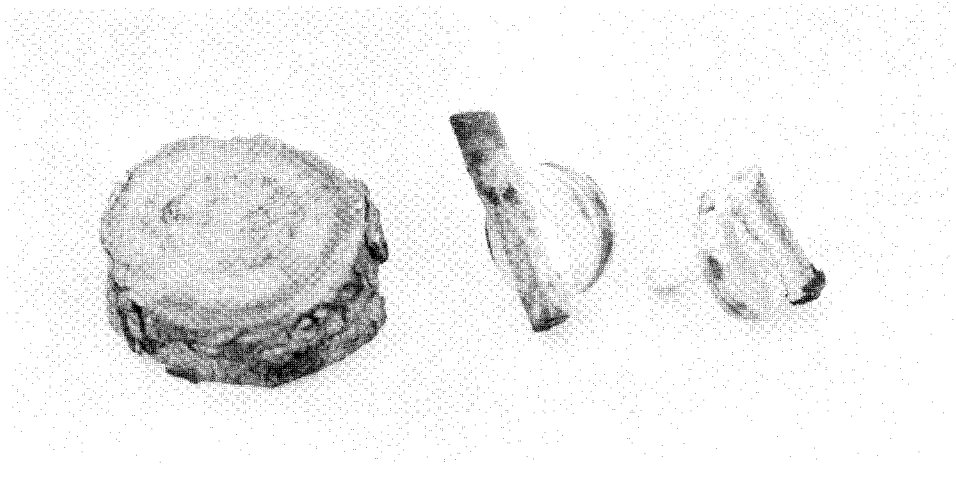

Figure 1. Specimens ready for SEM analysis showing full stem round of northern tree (left) and the two halves of more southern tree (centre and right). All specimens have been gold plated via vacuum evaporation.

chamber of the Cambridge 250T SEM. Considerable difficulty was experienced in pumping down the chamber because of the large specimen size and specimen porosity. It was left overnight (16 hours) under high vacuum after which it was sufficiently evacuated to proceed with SEM analysis and photography.

The microscope was set at one kv with a 400 milliamp beam current and a magnification of 50 x for photography. After locating the desired cross section for examination the specimen was sequentially photographed across its radius by photographing and then moving the specimen to the next desired frame using the x and y specimen manipulation knobs, until pictures of the entire radius were accumulated. Polaroid negative film was used. These pictures were then placed together by matching photograph over lap until a photo series of the entire radius was composed.

Although the results from the uncoated analysis at that time appeared surprisingly good, we felt that they would be appreciably better were the specimen coated before analysis. We were also troubled by the frequency of electron discharge lines across the photograph which we ascribed to the bubbling tree resin on the specimen surface. Resin bubbles can be seen on Figures 2, 3, 5 and 6.

After gold coating the specimen in a vacuum evaporation chamber, it was again

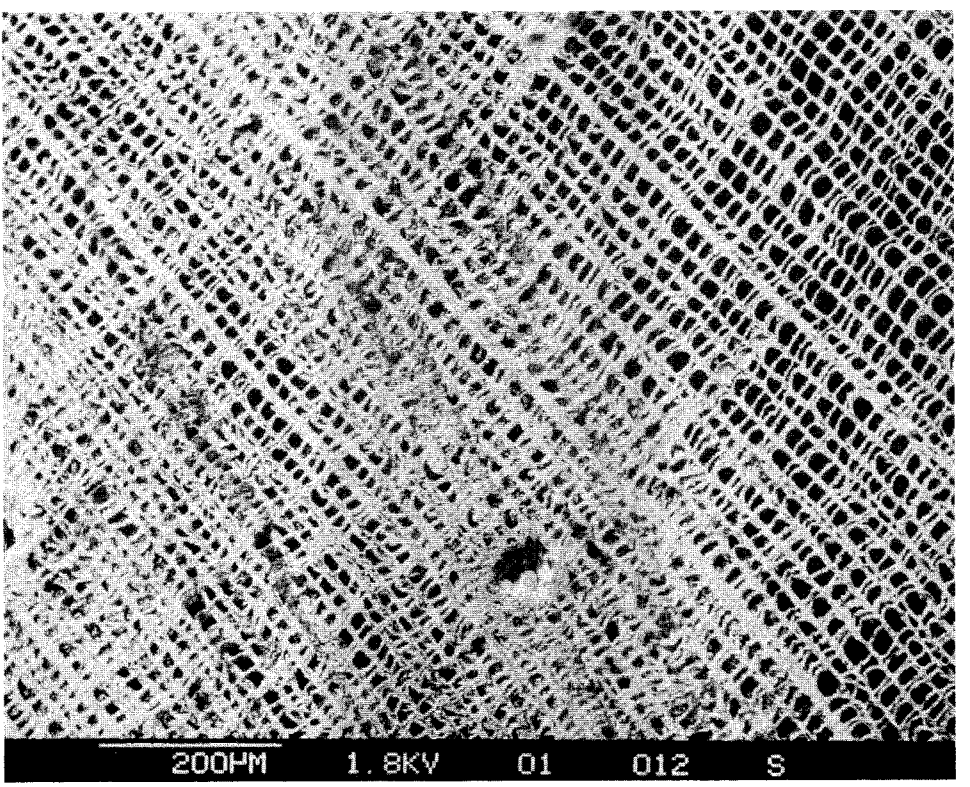

Figure 2. SEM micrograph of more northern tree specimen. Note ultra sensitivity of tree rings. These rings were unrecognizable using standard microscopic analysis. Circle in lower centre is a resin bubble caused by melting of resin due to the extreme heat of the electron beam.

placed in the SEM. The SEM was set at 1.8 kv with a magnification of 100 x and the entire photographic procedure repeated with greatly improved results (Figures 2 and 3).

The southern tree core specimen was prepared in a similar manner although no time was spent with an uncoated specimen. Two notable differences should be commented upon. Firstly, as the specimen was too long to fit on a single SEM stub it had to be cut. To allow for picture matching between the two halves of the core, the core was cut at an angle so as several of the same annual rings were represented on each half. The second noteworthy point is that because the specimen was much smaller, the time required to achieve the necessary vacuum for SEM analysis was greatly reduced. This core was photographed at a magnification of 50 x at 5.1 kv setting (Figures 4, 5 and 6).

I have presented a rather detailed account of the development of the technique indicating the time consuming approaches that Mr. Veto and I took. I will now present a summary of these after they were subjected to a critical path analysis.

SUMMARY OF TECHNIQUES

1) Select specimen to be analysed and cut a smooth surface across specimen with fresh disposable scalpel, razor blade or glass knife. For tree cores requiring more than one stab, cut across core at an angle to allow photocorrelation with rings.

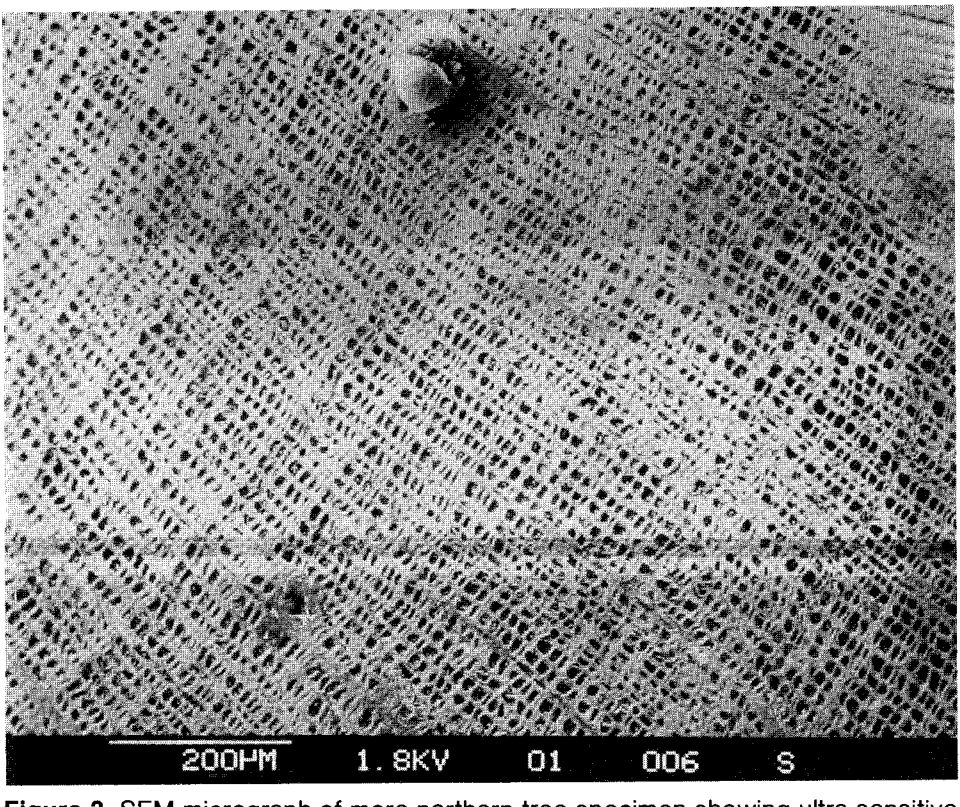

Figure 3. SEM micrograph of more northern tree specimen showing ultra-sensitive rings and resin bubble. Darker coloured lines across lower third of plate are a result of electron discharge. Scratches on upper right hand corner illustrate an area of specimen sanded with 400 grit paper and not subsequently cut with sharp scalpel.

2) Mount on SEM metal stub using silver based adhesive and place in warming oven under low vacuum.

3) Remove and place specimen or specimens in vacuum evaporation chamber and leave for one to four hours depending on specimen size. The larger the specimen, the greater the time. Vacuum evaporate and plate specimens with gold.

4) Remove specimens as required and place in specimen chamber of SEM and evacuate to desired vacuum.

5) Take sequential photographs of rings at magnification desired. Allow sufficient photo overlap for frame matching.

DISCUSSION

The principle advantages of this scanning electron microscope technique for tree ring analysis rest largely with the ease of sample preparation, the fact that it lends itself to batch handling of cores throughout all stages except actual specimen analysis, that cores may be examined without destroying them, that, since photographs are only taken of the surface cells of the core, the angle at which the rings line relative to the electron beam does not affect the clarity of the photograph, (a problem associated with x-ray densiometric analysis), and that no staining techniques are necessary for image enhancement and the core may be examined on the SEM display screen to select the appropriate magnification and picture area before the photographic exposure is

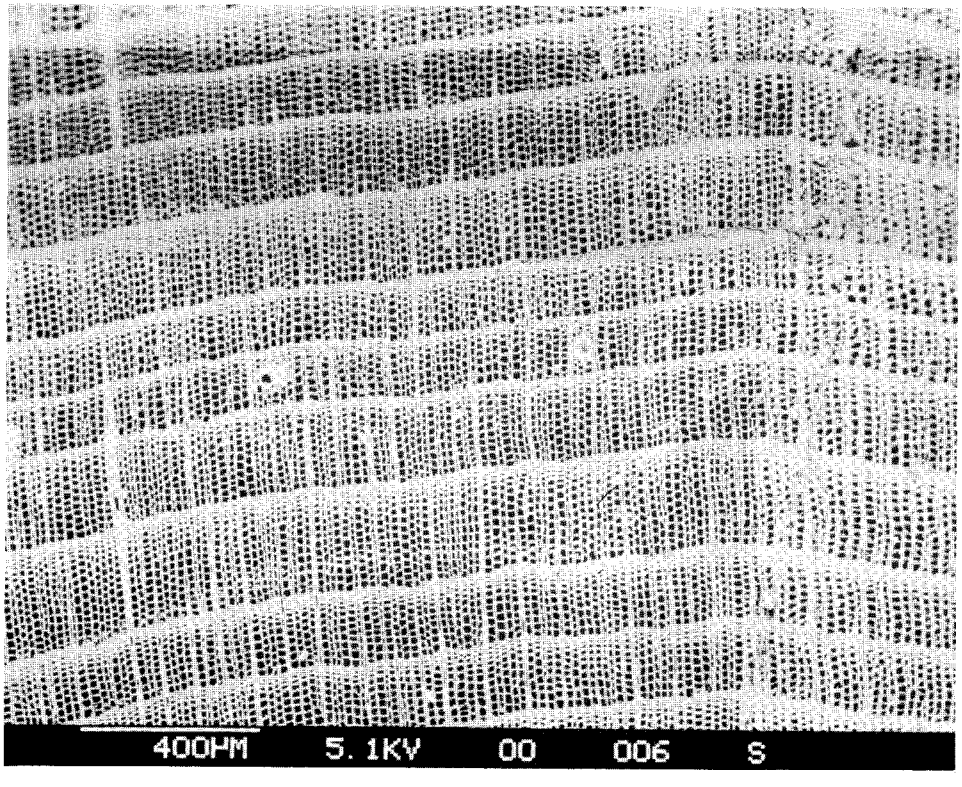

Figure 4. SEM micrograph of more southern tree core. Note well differentiated early and late wood in comparison with Figures 2 and 3. Out of focus area on right hand side of micrograph is a result of curvature of tree core.

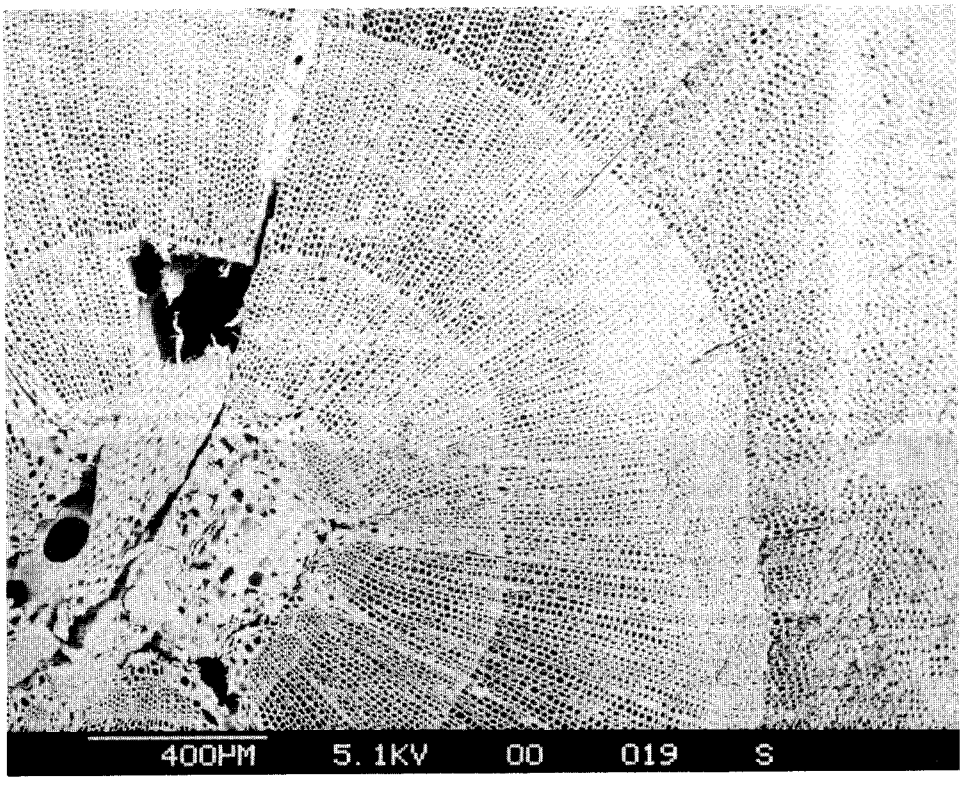

Figure 5. Micrograph of more southerly tree core showing good initial growth with well defined early and late wood.

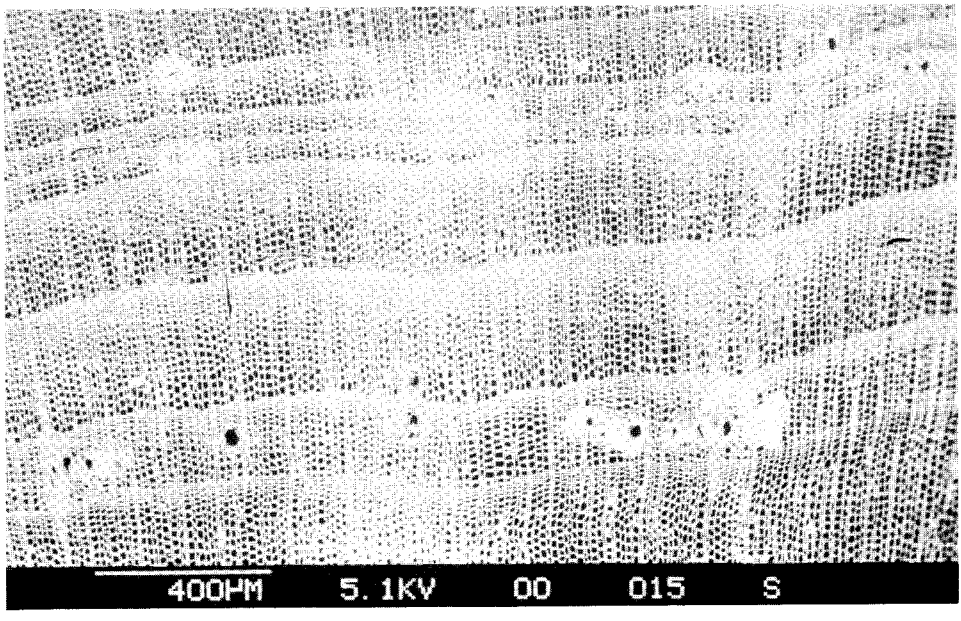

Figure 6. Micrograph of more southerly tree core showing sensitive rings.

taken. The use of polaroid negative film provides a particular advantage in that a print was available immediately. Although standard black and white negative film could be used at a somewhat lower cost, the benefits of having a polaroid print far outweighed the slight increase in cost.

It would appear that although this technique holds considerable promise for tree-ring analysis it is not by any means a panacea for problems in the field. It is only another tool to help the researcher put together the pieces of the puzzle when reconstructing tree-ring chronologies and in interpreting past climates from small sensitive trees at or near their ecological limits of tolerance. To be sure it can be used on large tree cores although this would appear to be a case of overkill unless some relatively specific aspect of that core were being studied.

Finally, as photographic negatives are produced in the process it may be possible to subject these to densiometric analysis thus gaining further benefits.

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