# RESPONSE TO PROGRESSIVE WATER STRESSING OF THREE ACTINORHIZAL SPECIES OF ALDER

By

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Bachelor of Arts

Hendrix College

Conway, Arkansas

1978

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1982

# RESPONSE TO PROGRESSIVE WATER STRESSING OF THREE ACTINORHIZAL SPECIES OF ALDER

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# ACKNOWLEDGMENTS

I wish to express my deepest gratitude and appreciation to the following:

To Dr. Thomas Hennessey for his invaluable guidance and unfailing support.

To Dr. Ronald McNew for so generously giving of his astute statistical acumen.

To Dr. Helen Vishniac for her sharp insight and equally sharp wit.

To Dr. Clark, Mr. McGill, Mr. Meriweather, and all the other friends of Hendrix College, for their wisdom and support.

Thanks to Floyd Brown for teaching me the ways of the computer, to Glen Hartman for his coordination and maintenance of the Controlled Environment Research Center, and to Eualetta Wilson, Julie Clifford, Shawn McWilliams, and Ed Lorenzi for helping me conduct the experiments.

Special thanks to Bob Heinemann and Roger Stewart for their moral support and for pitching in when truly needed.

Praise to God and to my family.

Funding for my research assistantship and support for this study was provided by the McIntire-Stennis Research Grant 1719.

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# CHAPTER I

# INTRODUCTION

## The Need

It should be no surprise to anyone that with the ever increasing demand for food and fiber there would follow a serious problem in supplying the nitrogenous fertilizers required. The need for fixed nitrogen is increasing at a substantially greater rate than population, simply because of the impact of economic growth on dietary habits (34). The United States Forest Service has projected that southern forest lands will need to double their production within the next 20 to 30 years if expected demands by the nation for wood products are to be satisfied (81). This is likely to create serious nitrogen depletion of forest soils. Indications are that the future demand for nitrogen will remain strong and, under current inflationary forces, exceed the forecasted levels (36). Dr. Raymond Ewell, a world authority on fertilizer needs, recently said: "The current world fertilizer shortage will continue indefinitely, perhaps for the rest of human history" (50, p. 642).

In order to meet their objectives, forest managers are pursuing a variety of management alternatives in an effort to grow more fiber on less land in a shorter time. Operationally, this oftens translates into shorter rotation periods which has a most significant effect on the productivity of the site. Nutrient removal is accelerated by the shorter rotations, especially in the case of total tree harvesting. The degree of soil depletion is directly related to the inherent soil fertility and the rate of nutrient replenishment. In Oklahoma, the forest soils are characteristically low in available nitrogen. Therefore, the trend toward shorter and shorter rotations will likely have serious impact on these sites (19, 30). To maintain these as productive sites under short rotation management, it will be necessary to replace nutrients at a greater than normal rate. Replacement of nitrogen will be essential if sustained productivity is to be maintained in the forest ecosystem.

Nitrogen requirements are provided by several sources, including mineralization of organic nitrogen in the soil, nitrogenous substances in rainwater, fixation of atmospheric dinitrogen by microorganisms, and by applying organic or inorganic chemical nitrogen fertilizers (36). One way that forest managers attempt to resolve the problem of maintaining site productivity while still meeting production schedules is through the application of commercial fertilizer. The primary source of nitrogen is in the form of ammonia, which is catalytically created under elevated temperature and pressure through the Haber-Bosch process (74). This is, in turn, combined with carbon dioxide to form urea. Historically, the raw materials used to supply the carbon and hydrogen have been petroleum. A major problem facing the industry is the shortage of such materials. As over 88% of the world production is based on hydrocarbon feedstocks, the rapid development of the recent energy shortage has had a major impact on the ammonia industry (74). Indeed, energy has received the most visibility with respect to the cause of increased fertilizer prices.

Aside from the energetics required to produce a commercial fertilizer, there are associated problems with the current technology of application. Of that which is applied, only 50% of the fertilizer nitrogen is utilized by the crops in the year of application; the remainder suffers biological transformations, losses by leaching, or losses as nitrogen gas to the atmosphere (31, 50). The effect is that half of the applied fertilizer never reaches the root zone. Also, timing of the application is necessary to avoid competition from understory weeds and to maintain high wood quality. Finally, for the entrepreneur, there is a significant time lag between the initial investment (application of fertilizer) and the realization of additional wood in the marketplace.

## An Alternative

Another route available in this ever-intensive search for new sources of nutrient nitrogen could be to capitalize on the fact that there are several woody plants, both legumes and non-legumes, that biologically fix atmospheric nitrogen into a form utilizable by plants. The National Research Council Report of the Committee on Research Advisory to the U.S. Department of Agriculture in 1972 identified biological nitrogen fixation as an area where sufficient support has not been provided. The report stated that productivity of field crops frequently is limited by the supply of available nitrogen and that examination of the nitrogen cycle in nature indicates that biological fixation of nitrogen accounts for over 50% of the fixed nitrogen currently available to plants (50). Incorporation of these plants into a silvicultural system of one type or another might be an efficient means of supplying additional nitrogen to developing forests through gradual addition to a developing biomass sink. A continuous net uptake by the forest would act to maintain a low concentration of soluble nitrogen in the soil which would be favorable to sustained fixation and reduce that lost by leaching (73).

The growth benefits of symbiotic nitrogen fixation to forest trees have exceeded those which could be obtained with conventionally applied fertilizer (26, 32). Nitrogen fixing plants have been supplied as nurse plants in the forest understory (27), as interplanted or mixed plantations of commercial species (18), by crop rotation, and by continuous cropping techniques (2, 19, 20, 33). The economic advantages of commercial fertilizer are, in any case, overshadowed by the aforementioned energy cost of its production (3, 64, 77) and by the likelihood that food production will have priority over fiber production in future competition for limited supplies of nitrogenous fertilizer.

The dinitrogen fixing symbiosis which occurs in the greatest variety of terrestrial ecosystems involves diverse genera of woody dicotyledonous plants which form nodules containing _Frankia_, an actinomycetous bacterium. It is now known that over 160 species, distributed among 15 genera, 18 families, and 7 orders of angiosperms, are capable of becoming nodulated with _Frankia_ (7). Exploitation of the woody angiosperms-_Frankia_ (now referred to as actinorhizal plants) dinitrogen fixing symbiosis is in its infancy compared with the wide and profitable use of legume-Rhizobium systems. Development has waited upon the recognition that actinorhizal plants are capable in at least some instances of fixing atmospheric nitrogen at rates comparable to those of nodulated legumes (15, 84, 85) and upon the control of the symbiosis made possible by the growth of _Frankia_ species in pure culture (12). Empirical investigations have demonstrated that the manipulation of actinorhizal systems could benefit man in a variety of ways related to forestry, land reclamation and stabilization, wildlife and stock feeding, or the production of biomass (2, 3, 18, 19, 20, 23, 26, 27, 28, 30, 31, 33, 45, 64, 77). At least 35 species of actinorhizal plants have been set out for reclamation and stabilization purposes, some quite extensively (23). The potential of species such as the European black alder (Alnus glutinosa) for growth on spoils has been demonstrated (25, 61), but there has been little or no selection of tree seedlings and only preliminary attempts to establish specific strains of endophyte in the root nodules to provide optimal performance (33). For many species, there is little information beyond that establishing their ecological importance as pioneer invaders of alluvium, moraines, sand dunes, alpine scree, road cuts, and eroded areas (45).

The greatest role legumes have played in the United States is that of food and forage. They do not provide fiber products, since woody legumes are largely of a tropical nature. The use of legumes is also restricted to well-watered agricultural lands, i.e., to those regions providing the climatic and edaphic conditions required by this family of host plants for growth and nodulation. On the other hand, actinorhizal plants occur in a variety of ecosystems which are unsuitable for nodulated legumes such as the many forested, stripped, eroded, arid and semi-arid, depauperate agricultural and non-agricultural areas for which productivity is limited not only be available nitrogen but by seasonal or constant water stress.

The extensive investigation of the legume-Rhizobium symbiosis has given a model through which the actinorhizal system can be profitably explored. For example: it is evident that the infectivity and efficiency of dinitrogen fixation depends upon the interaction between host and bacterial genotypes (11, 17, 65) and that interactions between the bacteria and soils are equally important in leguminous systems. Endogenous strains of bacteria are adapted to compete and survive in any given soil. Infective and effective strains of Rhizobium cannot compete or successfully colonize unless they are also site-adapted (56). Therefore, those native strains producing a preponderance of nodules may be ineffective dinitrogen fixers (29). A limited number of recent reports indicate that the infectivity and efficiency of Frankia strains also depends upon the interaction with the host genotype (33) and that individual soils may lack infective strains, or may contain infective but inefficient strains (1). The enhancement of the productivity of actinorhizal plants in water-stressed sites will require the surveying of a broad range of Frankia isolates not only for their infectivity and efficiency in a variety of host genotypes, but for their adaptation to the edaphic conditions of specific sites.

Climatic limitations of biological nitrogen fixation have been noted, but little explored. Symbiotic fixation is correlated with photosynthate supply to the root nodules, with temperature and with moisture availability. Though moisture and temperature stress are thought to be the primary environmental factors responsible for the relative lack of nodule formation on legumes in the grasslands and deserts of the southwest United States (45), and are frequently concomitant in semi-arid areas, current knowledge of the fundamental physiology of biological nitrogen fixation has not provided the means of alleviating the effects of these stresses. The factors limiting moisture stressed actinorhizal plants are virtually unknown. Although the rhizobial system provides a model for studying them, actinorhizal systems do not follow it closely. According to Sprent (71, p. 305), "the prime effect of water stress on (rhizobial) nodules (is) to depress oxygen intake" and hence, respiratory production of ATP required for dinitrogen reduction. Fixation in actinorhizal systems is also correlated with oxygen tension (5), but the actinorhizal nodule, unlike the rhizobial nodule, is essentially open to the atmosphere (78). The fact that legumes fail to nodulate in the southwestern United States (45) suggests that, though typically profligate in their water usage (71), legumes are more xerotolerant than their nodules or symbiont. The effects of temperature and water stress are usually somewhat confounded in these field studies. The maximum optimal temperature reported for rhizobial fixation is 28 degrees Celsius (83). Dinitrogen fixation by nodulated legumes unstressed by temperature is maximal at the moisture provided by field capacity (57). <u>Cystisus</u> <u>scoparius</u> (Scotch Broom), a rhizobial legume, ceases to fix nitrogen at stress greater than a water potential of -5 bars (38). Actinorhizal plants have been found to successfully fix nitrogen in temperatures ranging up to 32-36 degrees Celsius (39). Actinorhizal <u>Purshia</u> from a dry site in Oregon maintained relatively high fixation rates at moisture stress up to -24 bars (16). Actinorhizal <u>Ceanothus velutinus</u> performed well up to -20 bars (51). The rhizobial nodule suffers irreversible damage when it loses 20% of its fresh weight; the actinorhizal nodule must drop below 60% of maximum fresh weight to suffer the same damage. Whether it is the character of the host plant, the <u>Frankia</u> symbiont, or the nodule morphology that is of primary importance in moisture stress resistance is not yet fully known. It is apparent, though, that the actinorhizal systems are clearly different.

## The Intent

There has not been a research breakthrough in either the field of chemistry or biology that has produced a new technology for nitrogen input into crops during the last 70 odd years (34). It was in 1904 that Haber discovered the catalyst which led to the development of the Haber-Bosch process for industrial fixation of dinitrogen gas. The existence of non-legume nitrogen fixation was first recognized in 1896 by Hiltner (40), but the first scientific report of the importance of root nodule plants was by Dinger in 1895, when he reported the correlation between root nodule number and foliage nitrogen in *Alnus glutinosa* (68). However, as the plants were of little direct economic importance, they received scant attention until the early 1950's. Since then a considerable but rather disjointed amount of work has been done on the group.

A series of research projects are underway on this campus spearheaded by Dr. Thomas Hennessey of the Department of Forestry, Oklahoma State University, and Dr. Helen Vishniac of the Microbiology Department, of which this paper is a small part. It involves the investigation of the effects of water-stress on dinitrogen fixation by actinorhizal *Alnus* species as a function of the xerotolerance of the host and endophyte. The desirability of improved xerotolerance in actinorhizal *Alnus* is stressed by silviculturists: "the ability to survive and fix nitrogen . . . under water-stress . . . (is) a characteristic that must be selected" (59, p. 244), "other important traits (in genetic improvement of alder) will include moisture-stress tolerance" (33, p. 120). Yet, neither the water requirements of the alders as species nor the intraspecific variation in response to moisture stress has been established under controlled conditions. Neither has the response of *Frankia* to medium osmolality been determined. Previous studies of actinorhizal plants dealt only with the nitrogenase activity of nodules from field grown plants under seasonal climatic stress (15, 51). There are no reports of studies using actinorhizal plants under environmental or genetic control, particularly when considering the xerotolerance of the host plant and symbiont individually before studying them in a symbiotic relationship.

The genus *Alnus* includes species of marsh and sandy waste, disturbed stream bank, and dry lava slopes (68, 69). They are suitable for land reclamation and amenity planting (23), as well as timber production (19, 20). *Alnus* has the greatest direct commercial utilization of actinorhizal genera in the United States today, and the potential for use in more arid habitats currently inhabited by other actinorhizal plants. *Alnus* includes species with rapid, indeterminate growth and desirable wood properties which can provide a substantial economic return to landowners (4, 24). Techniques for seed germination, cloning, and for growth under controlled conditions are available for *Alnus* species. Also, numerous isolates of *Frankia* capable of infecting *Alnus* are now available. *Alnus*, therefore, is the genus of choice as a model (4) for investigations into the relative contributions of host, *Frankia*, or nodule to xerotolerance in dinitrogen fixation by woody plants. If species belonging to other genera should later be required for particular applications, this investigation could provide a model for fitting the actinorhizal plant to a selected environment.

The development of alders of improved xerotolerance requires, as the first order of study, the establishment of a base line of response to water stress for alder species of a determined clonal lineage lacking symbionts (but fertilized) in a controlled environment. This is the specific task of the present paper--the methods of which will be elaborated upon in a later section. It is expected that this study will be followed by the work of other researchers, who will be involved with the determination of xerotolerance and its interstrain variation in Frankia, and the investigation of the effects of water stress on dinitrogen fixation in actinorhizal Alnus for which the degree of nodulation, the infectivity and comparative efficiency of the endophyte, as well as the genetic history of both host and endophyte are known and standardized. The relative contributions of host, endophyte, and nodule physiology to xerotolerant dinitrogen fixation should then become clearer, a desideratum before entering upon laborious and time-consuming Alnus breeding programs for this character.

# CHAPTER II

# LITERATURE REVIEW

## Plant-Water Relations

Successful growth of trees requires adequate water as well as mineral nutrients, enough oxygen in the soil for roots to function effectively, enough light for photosynthesis, and a favorable temperature. Water is essential to the life of trees, often comprising as much as 90 to 95% of the fresh weight (46, 82). Water provides certain plant parts with their mechanical strength through the mechanism of cell turgor, since much vegetative tissue is composed of cells with non-lignified walls. Such plastic bodies become mechanically stable under the influence of the hydrostatis pressure developed. Water is a raw material in metabolism and synthesis reactions that occur in living trees, even though this amounts to a small fraction of the total amount of water that passes through the tree. The greatest amount of water moves into the roots, through the stem, into the leaves, and out into the atmosphere in what is known as the transpiration stream (14, 41, 46, 82, 86).

Trees are capable of transpiring large amounts of water due to an extensively exposed leaf area. It is thought that forested areas of the southern United States may transpire up to 1200 liters of water per hectare per day (44). Trees usually exhibit a daily internal water deficit due to such a high rate of water loss through transpiration. Water deficits can be caused by either excessive loss of water in transpiration, slow absorption from the soil, or perhaps most often, a combination of the two (46). Deficits caused by excessive transpiration alone are common, but usually shorter and less severe than those caused by inadequate absorption. However, if soil moisture is not replenished adequately, these temporary water deficits will tend to persist longer each day as leaves lose their capacity to recover turgidity at night.

Water deficits affect tree growth by modifying the rates of various physiological processes and conditions which control growth. Indirectly, growth is reduced by interference with various metabolic processes such as photosynthesis and nitrogen metabolism, and by reduction in processes such as translocation and salt absorption. Growth is reduced directly because loss of turgor decreases cell enlargement. Water deficits not only reduce the amount of growth but also change the character of growth, as seen in the thicker leaves and earlier change from spring to summer wood in trees subjected to early summer droughts (46).

A water stress may conceivably arise either from insufficient or excessive water activity in the plant's environment. In the case of terrestrial plants in nature, the former occurs as a result of a water deficit or drought and therefore is called a "water deficit stress" (shortened to water stress) or drought stress (47, 49, 70). Drought is a meteorological term, and is commonly defined as a period without significant precipitation (49). Since this lack of precipitation must lead to a water stress, the term "drought stress" is defined as a water stress, or more simply, a natural water stress as opposed to an artificially induced water stress. However, the two terms (water stress and drought stress) are often used interchangeably. The term drought stress is less ambiguous than water stress, since it can only refer to a deficit, never to an excess, of water.

## Evolution of Methodology

Early methods of controlled drought studies were often quite crude in design. They usually led to the death of the subject plants, often a criterion in measuring drought resistance. For example, in an early report, Shirley (66) quoted one N. A. Maximov as saying the best measure of the drought resistance of a plant was its capacity to withstand permanent wilting. Accordingly, Shirley described a device for testing drought resistance of trees. This mechanical contrivance consisted of a thermostatically-controlled, illuminated plant chamber with a calcium chloride desiccator supplying dry air. It was assumed that the severity of the drought conditions were, with other conditions constant, a function of the evaporative power of the air or saturation deficit. Seedlings were selected and potted in tin cans with fine sand. Allowing two to four weeks for the plants to become established, the cans were watered to a definite moisture content, sealed in paraffin, and placed in the desiccating chamber. Plants were tested at ambient temperatures of 35 to 40 degrees Celsius, a temperature which the plants were apparently able to initially withstand, even with low humidity. However, the machine was run continuously until all plants were dead. Time until death and soil moisture content at death were used as the criterion for drought resistance.

A sample run utilizing this procedure was described by Shirley (66) using three classes of seedlings of white spruce (<u>Picea glauca</u>). These classes were two years old (2-0), three years old (3-0), and three year old seedlings which had spent one year in the transplant bed (2-1). The ambient temperature was 38 degrees Celsius and the relative humidity about 10%. The soil moisture at death was determined to be about 14% for all pots. The 2-0 and 3-0 seedlings lived 20 and 21 days, respectively, while the 2-1 stock lived only 13 days. Shirley (66) concluded that the transplanted stock was less drought resistant than either of the other two classes of seedlings.

Further use of Shirley's (66) plant desiccator was reported in a work by Meuli and Shirley (53) testing for drought resistance in green ash (Fraxinus pennsylvanica var. lanceolata (Borkh. Sarg.) seedlings. Seedlings of seed collected from 83 trees distributed among 39 locations in five plains states (North Dakota, South Dakota, Nebraska, Kansas, Oklahoma) were grown in the greenhouse for six months to one year and then subjected to Shirley's "death test" for drought resistance. The seedlings were ranked according to their time of death and these numerical scores were averaged for each designated region to obtain a mean score. These scores were used as a criterion for rating drought resistance.

Grouping the seedlings according to region was found to be statistically significant, with three distinct regions being established by Meuli and Shirley (53). Seed from the northwest region produced the most drought resistant plants, while plants from southern and eastern seed were least resistant. The differences within each region were too slight to be considered significant. Differences were also noted in size of seedlings, color of foliage, and time of resumption of growth in the spring. These differences led to the suggestion of possible climatic races of green ash due to generations of natural selection.

A third use of Shirley's (66) drought stressing techniques was reported in work by Shirley and Meuli (67) on pine. The particular species used in the tests were red pine (Pinus resinosa Ait.), eastern white pine (Pinus strobus L.), and jack pine (Pinus banksiana Lamb.). The tests were run at ambient temperatures of 35 to 37 degrees Celsius with a relative humidity of 20%. The plants were placed in the drought machine immediately after potting in a soil of predetermined moisture content. They were allowed to remain without additional water until death, which was determined by when the needles became brittle to the touch. It required four to seven days for the seedlings to die, whereupon it was determined that they retained a moisture content of around 10% based on dry-weight. Certainly, with such low moisture content the seedlings were quite dead, but question arose as to just how long they had been dead. This question was echoed several years later by Brix (10), who reported that the leaf-moisture content of 110%, based on dry-weight, indicated a critical plant-water balance in loblolly pine (Pinus taeda L.), below which seedlings did not recover even after rewatering. Another study of loblolly pine by Ursic (80) found that when leaf moisture was reduced to 80%, leaf turgor was never regained. Also, one might add that hardening off of the plants during a longer drought period might have changed the outcome of the very short, severe tests conducted by Shirley (66).

A study of Ferrell and Woodard (22) found that proper determination of the behavior of Douglas fir (Pseudotsuga menziesii Franc.) after natural drought required more than a "few days." Douglas fir seedlings were planted with sunflower seeds in pots of clay loam soil. When the sunflowers had grown three pairs of secondary leaves, the drought period was begun. At the first sign of permanent wilting, the sunflowers were cut off at the soil surface and the pots of Douglas fir seedlings were placed under the selected environmental conditions to undergo drought hardship and death. Two types of tests were made to determine ability to resist death under severely dehydrating conditions. One set of pots were sealed in paraffin. With such an arrangement, transpirational and root absorption differences would be most important in determining seedling survival. Another set of pots were covered with a light sand mulch to slow but not stop evaporation. With this method, differences in survival were due to internal tolerance of the seedling to extreme desiccation. The results revealed there to be regional differences in the response of Douglas fir to drought. Dry site (inland) seed sources were found to be more drought resistant than wet site (coastal) seed sources, although local variability (north versus south slope) was also present.

A follow-up study was conducted by Pharis and Ferrell (60) using two measures of drought resistance of Douglas fir: time until death and soil moisture content at the death point. The surface of all pots was covered with sand so that evaporation from the soil accounted for only 10% of total water loss. The results confirmed the work of Ferrell and Woodard (22). Inland sources of Douglas fir were more drought resistant than coastal sources in Oregon. Pharis and Ferrell (60) also indicated that needle-moisture content might be a feasible index for determining the coniferous seedling viability, except near the "death point." Seedlings were classed into coastal and inland groups according to their needle-moisture content under well watered conditions.

In these earlier works the so-called "death test" method ran into a few obstacles. There was difficulty in determining the exact time of death, for one. Ferrell and Woodard (22) found great difficulty in determining the time of death in Douglas fir seedlings with greater accuracy than a day. The severity of the drought augmented the difficulty encountered in assessing the viability of the plant. Brix (10) addressed this problem by noting that it is not the death of a certain organ, such as leaves, but rather the ability or inability of the plant as a whole to recover after being severely dehydrated which should be studied. He concluded that an accurate method for determining this threshold is of prime importance for comparisons of plant responses to simulated drought conditions.

With this in mind, the search was on for more sound methods and better parameters to measure. As drought investigations advanced, the short, severe "death test" approach was abandoned for more quantitative methodology which emphasized measurement of physiological parameters.

The vague "droughty conditions" of earlier works was upgraded to the more specific "soil or plant moisture stress," followed by a description of standardized quantitative methods. Transpiration, water potential, and leaf turgidity measurements dealing with the internal water balance of plants replaced the nebulous "time until death."

Under conditions of variable moisture stress, Zavitkovski and Ferrell (87) determined rates of photosynthesis, respiration, and transpiration in Douglas fir seedlings. Seeds were collected from wet and dry sites, potted in sandy soil, and allowed to grow for two to three months. The seedlings were then transferred to a growth chamber where conditions were maintained at a 35 Celsius day, 20 Celsius night, and 16 hour photoperiod. The seedlings were dehydrated to various degrees by withholding soil moisture before measuring. Photosynthetic rates were found to decline sharply after soil moisture stress of one atmosphere was reached, with no major differences between seed sources. Respiration and transpiration rates were higher overall, and variability of relative turgidity greater, for the wet-site seedlings. The dry-site seed source had higher photosynthetic rates at higher relative turgidities of the leaves and lower rates at lower relative turgidities. Drought resistance of the dry-site seedlings effectively prevented excessive water loss and prevented relative turgidity from dropping below its level found at well-watered conditions.

The effects of soil water stress on potted seedlings of *Eucalyptus rostrata* Schl., *E. polyanthemos* Schau., and *E. sideroxylan* Cunn. var. *rosea* Hort. were studied by Quraishi and Kramer (63). Seedlings were grown in a greenhouse in sandy loam soil. After they were well established, the pots were enclosed in plastic bags which were tied around the stems to prevent water loss by evaporation from the soil. Each species was divided into three groups: controls, moderate, and severe stress levels. Water stress was created by withholding water until a certain degree of wilting took place. The controls were kept at field capacity. Transpiration, shoot growth, and leaf growth were reduced most by water stress in *E. rostrata* and least in *E. sideroxylan*. Leaf water deficit at stomatal closure was greater and leaf water potential (at wilting point of sunflower plants) was lower in *E. rostrata* than in *E. sideroxylan*. Excised twigs of *E. rostrata* also lost water more rapidly than twigs of the other two species. Rate of root extension did not differ significantly among the three species. Quraishi and Kramer suggested that *E. rostrata* suffered more injury from soil water stress than the other two species, due to its stomata closing more slowly when stress developed and because it had a larger leaf area resulting in a higher and more rapid rate of transpiration.

Townsend and Robert (79) compared red maple (*Acer rubrum* L.) seedlings grown from open-pollinated seed of wet and dry sites by subjecting them to three levels of plant water stress. The seedlings were treated in much the same manner as Quraishi and Kramer (63) dealt with theirs. Transpiration was greatest for seedlings from the wet site at both low and high water potentials. Growth rates were also greater for the wet site than for the dry site seedlings. However, wet site seedlings came to temporary and permanent wilt sooner than dry site seedlings, which suggests that the dry site seedlings are more capable of better continued growth under drought conditions.

Ladiges (48) designed an experiment for four varieties of *Eucalyptus viminalis* Labill. to compare: (1) the drought tolerance of six-month-old seedlings; (2) the drought tolerance of three-month-old seedlings; (3) the relation between leaf water content and water potential under drying conditions. Seedlings from two wet and two dry sites were divided and treated as mentioned in the preceding papers for the first two experiments. Another set of seedlings for the third experiment were grown in a selected field setting from which shoots were collected for measurement. Six-month-old seedlings of the two dry sites showed greater resistance to desiccation and were less damaged by sustained drought than seedlings from two wet sites. The more resistant seedlings did not simply avoid drought by quickly closing stomata, but were capable of maintaining relatively high rates of transpiration under moderate moisture stress. The set site seedlings, on the other hand, wilted at a higher relative turgidity of leaves than did those from the dry sites. It was suggested that the greater tolerance of the dry site seedlings to drought is related to physiological resistance of the protoplasm to desiccation.

A comparison of yellow poplar seedlings subjected to a range of moisture stresses was studied by Pope and Madgwick (62). One set of seedlings were potted and brought up to field capacity where they were kept until new growth was evident. Then they were broken into groups: controls, which were maintained at field capacity, the rest being subjected to varying percentages of field capacity (80, 60, 40, 20%), depending on treatment. Another group of seedlings were grown in hydroponic media containing Hoagland's colution. After new growth was evident, all plants were raised in osmotic stress to 0.3 bar by the introduction of polyethylene glycol, and inert osmotic, stress-producing agent. Later, the seedlings were assigned to stress treatment of 0.3, 0.5, 1.1, 2.4, and 8.4 bar, respectively. These values related comparatively to the stress levels of the potted seedlings. The two separate experiments indicated that total dry matter production, stem height and leaf area, decreased with increased moisture stress. The number of leaves produced was unaffected by stress, except at the most severe stress. The number of senescent leaves increased as moisture stress increased. The allocation of new growth was unaffected by either moisture stress or time and decreased in order of root, foliage, and stem for the soil grown plants and foliage, root, and stem for the solution grown plants.

Citing a lack of work on the effect of water stress on nodulation, Kant and Narayana (43) proceeded to subject seedlings of Casuarina equisetifolia, an actinorhizal tree, to different degrees of water stress. Seedlings were raised in pots using unsterilized field soil. One group of pots was watered to field capacity daily. A second group was watered on alternate days, and a third group on every third day. The root systems were inoculated with a suspension of crushed nodules. After 180 days (six months), the plants were harvested. The length of shoot and root, wet and dry weight of shoot and root, and the nodule number, size, and wet and dry weight were taken. The lengths of weights of the shoots and roots and weight of nodules were found to decrease with increasing stress along with a decrease in nodule size and number. There was no degeneration of nodules nor cessation of nitrogen fixation when soil moisture was replenished once in 72 hours. It was suggested that the reduction in number of nodules at different levels of water stress could have been the result of minimum opportunity for infection, due to the soil moisture condition.

In the more recent literature (6, 13, 21, 44, 58, 76) there has been a considerable interest in stomatal physiology and the quantification of the diffusive resistances of stomata to water vapor movement by researchers in plant-water relations. During the past few years the water vapor diffusion parameter (42, 54, 55) has become a widely used tool for field studies of stomatal resistance, replacing, to a considerable degree, other types of porometers and related methodologies for quantitative investigations. The attraction of the diffusion porometer is that it measures the actual diffusive resistance of the leaf to water vapor movement, and the transfer processes and pathways that are involved are essentially the same as in natural transpiration. The reason for the emphasis on stomatal resistance becomes apparent from a perusal of the soil-plant-atmosphere continuum. The main pathway for gas exchange between plants and their environment is by way of the leaf stomata. Consequently, variations in stomatal aperture can markedly affect the transpiration rate. Resistance to water transport within the plant would reduce transpiration but also would cause desiccation of leaves and eventual death. Therefore, resistance within plants are to be considered potentially "harmful" while stomatal resistance is "protective" (37).

In a northern hardwood forest in central New Hampshire, Federer and Gee (21) subjected several trees to artificial moisture stress by trenching around them and covering the soil with polyethylene. After two to three weeks with rain excluded, soil-water potential ranged from -0.4 to -0.8 bar. Daytime abaxial stomatal resistances measured on yellow birch (*Betula alleghaniensis*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia* Ehrh.) had doubled, indicating significant stomatal closure caused by water stress. However, daytime xylem potentials on stressed trees (measured with a pressure bomb on twigs) were the same as or higher than those on unstressed trees. Stomatal control effectively prevented excessive water loss and prevented xylem potential from declining below its normal levels. This suggests that the trees had very good stomatal control of transpiration loss and could very likely tolerate a period of drought through increasing stomatal resistance.

Daytime changes in needle water potential and needle diffusion resistance of young *Pinus banksiana* (jack pine) and *P. resinosa* (red pine) were studied during a single growing season by Pereria and Kozlowski (58). As the season progressed, water potentials in the early morning grew lower, with a more evident decrease in the red pine. In addition, stomata of jack pine appeared to close earlier in the day. Jack pine's greater capacity to avoid drought was reflected in a lower rate of transpiration and slower soil moisture depletion associated with lower leaf area and stomatal closure at higher needle water potential.

As part of a comparative field study on thinned and unthinned stands of Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) Tan, Black and Nnyamah (76) investigated the relationship between stomatal resistance and environmental variables. During the daytime, stomatal resistance was mainly related to the soil water potential and the vapor pressur deficit of the canopy air. Stomatal resistance appeared to increase with a decrease in soil water potential and an increase in vapor pressure deficit. Dnring the study, there were two dry periods separated by a short period when the soil was completely rewetted. This provided the unique opportunity to study the importance of stress history in changing stomatal behavior. Stress history was found to have little effect on the relationships studied.

In a study by Clemens and Jones (13), potted plants of Acacia and Eucalyptus species were grown under different levels of shading, nutrition, and irrigation to assess the effect of these factors on plant water use. The two levels of shading employed were full sun and 50% shade. A liquid nutrient solution was applied at watering intervals in high, medium, and low concentration of 2.0, 0.5, and 0.125 g per liter. The three levels of irrigation were daily watering (high); watering only when leaves wilted (low); and a frequency (medium) judged to be midway in time between these two extremes. Water use was affected only by the irrigation treatment;

control plants that had received water daily used appreciably more water than plants repeatedly subjected to water stress. Water stress conditioning of plants reduced their water consumption demand because of a more pronounced tendency for stomata to close prior to wilting. Conditioned plants exhibited drought tolerance at lower leaf water potentials.

Stomatal control of gas exchange was investigated by Bennett and Rook (6) as a possible factor in explaining why two clones of *Pinus radiata* (Monterey pine) differed in their rate of transpiration and survival. The response of stomatal resistance, apparent photosynthesis, and transpiration rates were measured against increased evaporative demand. The response of apparent photosynthesis to carbon dioxide concentration was also measured. Clone 457, which had a low survival rate in the field, showed a transpiration rate up to twice that of clone 456, which had a high survival rate. The stomatal resistance of clone 457 was almost half that measured for clone 456 under all experimental conditions. The two clones showed similar stomatal response to increased evaporative demand, but the greater absolute values of stomatal resistance by 456 resulted in lower overall rates of transpiration in that clone. The measured response of photosynthesis to carbon dioxide concentration for clone 457 was twice that of clone 456. It was suggested that a factor in determining the relative survival rate of the two clones was the greater ability of clone 456 to limit transpiration water loss through greater stomatal resistance.

Kelliher and Tauer (44) used measurement of stomatal resistance to make a preliminary classification of the drought resistance of four clones of eastern cottonwood (*Populus deltoides* Bartr.). Clones were selected from natural stands, two from a dry site and two from a wet site. Following establishment in a greenhouse, cuttings were taken, rooted under mist, and potted with a time-release fertilizer. They were allowed to acclimate for nine days in a growth chamber while being equally watered. Just prior to the experiment the pots were sealed in plastic bags. The course of the project ran for three periods of nine days for a total of 29 days. The controls were given 360 ml water daily for the entire experiment. The moderately stressed plants were given 240, 225, and 210 ml water daily for each of the three periods. The severely stressed plants were given 120, 90, and 60 ml water daily for each period. Stomatal resistance, transpiration, leaf area, and height were measured on alternate days. Under the severe water stress level, the wet site clones produced more leaf area and ceased expansion of leaves later, at higher resistance values, than dry site clones. Height growth, conversely, was greater and persisted farther into the experiment for the dry site clones. Overall resistance values were considerably lower for the dry site clones than for the wet site clones. It was interesting to note that these differences in stomatal resistance between the plants from the two sites were discernible with or without the drought stress. Thus, it was suggested that screening for drought tolerance in cottonwood may be possible under common environmental conditions.

## Studies on Alder Stressing

In reference to actinomycetes nodulated plants, there are currently few studies dealing with the topic of water relations. There is a scant assortment of studies noting the xeric characteristics of a few actinorhizal plants, and there have been statements to the effect that more elaborate studies are needed in the area of water relations (33, 59), but published data specific to alders is lacking. In an early paper by McVean (52) dealing with the various British alder populations, it was suggested that stomatal control of transpiration was poor in Alnus glutinosa and that alders in general were rather lavish in their water use.

The influence of three different soil moisture conditions on transpiration, growth, and the transpiration ratio of several seedlings, including alder (Alnus glutinosa Gaertn.) were studied by Takahashi (75). One-year -old seedlings of alder were transplanted into specially prepared pots of native soil and maintained at three moisture levels: (W) soil moisture was kept at

93-82% based on maximum water-holding capacity, (M) moisture was maintained at 66-55%, and (D) soil moisture was maintained at 38-27%. The project spanned a little over three months at a temperature ranging no higher than 35 degrees Celsius with approximately 75% outdoor light intensity. Water was replenished daily with measurements taken every five days. After the course of the experiment, the leaves, stems, and roots were dried and weighed to determine the transpiration ratio. In agriculture, the amount of water used per unit of dry matter produced is useful in evaluating the efficiency of water use. This measure was formerly known as the water requirement, but is now usually termed the transpiration ratio (47). In this context, a high transpiration ratio value indicates less efficiency in water usage during growth, whereas a low value indicates better efficiency in water usage during growth. Alnus produced exceptional growth in wet soil with leaf area increasing with increasing dry matter production, whereas it was greatly inhibited in the dry soil condition. There was notable increase in top-root ratios with an increase of moisture. It was found that Alnus was more sensitive to the saturation deficit than the other species, and had the greatest differences in transpiration among the three soil moisture conditions. The effect of soil moisture on transpiration ratios was statistically significant for Alnus in wet soil moisture. The transpiration ratio was lowest for Alnus and had less variation among the three treatments as compared to the other four species in the project (Larix, Abies, Picea, Betula). This may possibly be a significant factor in the drought study of alders. Takahashi (75) found the transpiration ratio for Alnus ranged from 275 to 331 g $H_2O$/g dry matter. This compares favorably to three crop species familiar to Oklahoma where the ratio was found to range from about 200 to 300 g $H_2O$/g for alfalfa (47). It was suggested that Alnus had a low transpiration ratio due to nitrogen supplenentation by the Frankia infected root nodules. It is a known fact that fertilization and other cultural treatments which increase yields usually tend to decrease the transpiration ratio and increase the efficiency of water use (47).

Hari and Luukkanen (35) studied the effect of water stress, temperature, and light on photosynthesis in speckled alder (Alnus incana (L.) Moench) under controlled environmental conditions. Seedlings about 10 centimeters high were transplanted into containers of sandy loam soil. The experiment was conducted for two months in a greenhouse with about 60% of the natural light intensity available to the plant. Net photosynthetic capacity, light, temperature, and atmospheric water pressure deficit were continuously recorded. The potted plants were given daily predetermined quantities of water. At the conclusion of the experiment two different effects of water stress were found. When sufficient amounts of water were present, photosynthetic carbon dioxide fixation rates largely followed the variation in temperature and light. After prolonged water stress, though, higher temperatures caused a large decrease in net carbon dioxide uptake even if the plant apparently had sufficient water. The effects of water stress on photosynthesis were studied at a constant temperature as well as in conditions where all factors were allowed to vary. It was this latter approach that clearly demonstrated the overriding role of temperature in controlling the photosynthesis of plants under water stress.

Braun (8) conducted a series of comparative studies on *Alnus glutinosa* (L). Gaertn. (alder) and *Salix alba* (L.) Liempde (willow), dealing with growth and water economy. Large potted seedlings (223-341 cm tall) were grown under similar conditions for the length of one vegetative period. Measurements were made of basal area increment, stem growth and volume increment, number, area and volume of leaves, water consumption (absolute and per meter squared of leaf area), and water economy. The results illustrated that although the ecological demands of both species are very similar, there are fundamental differences in their physiology. The volume increment (stems and leaves) of *Salix* was very slightly larger than that of *Alnus*, but the consumption of water for the same leaf area was much greater. For the same water consumption Salix produced only two-thirds the biomass of Alnus, making the latter the more economic of the two.

In a second related study, Braun (9) extended the previous work with a study of the amount and seasonal pattern of growth, water consumption (in liters), and productivity of water consumption (liters per square meter of leaf area) in alder (Alnus glutinosa (L.) Gaertn.) and willow (Salix alba (L.) Liempde). Alders 90 to 120 cm tall and willows 230 to 260 cm tall were grown in containers for two growing seasons. Basal area increment, number and area of leaves, and water consumption were measured at four weekly intervals. Water uptake, foliar development, and basal area increment peaked at about mid season for both species. The number of shoots, amount of foliage, and water uptake increased considerably from the first to the second growing season, with basal area only slightly. Alder consumed less water in relation to leaf mass than willow, but basal area was about the same for both. It was found that both species could survive in a virtually oxygen-free substrate but water uptake and growth were reduced. Overall, alder proved to be more economical and showed greater vitality under difficult environmental conditions.

# CHAPTER III

# PROCEDURE AND RESULTS

## The Species Involved

There are 35 known species of alder in the world. They exist as trees and bushes distributed primarily in the north temperate regions of the world, but also in higher elevations in Central and South America, northern Africa, and Asia. Eight species native to North America reach tree size, although seven of them are more often large shrubs. Alders are generally found in moist, cool areas from sea level to 2400m. They often grow along the banks of streams, rivers, ponds, and swamps where they form dense thickets. Because of their dense growth habit and their role in increasing the level of valuable nitrogen to the soil, alders are important as erosion-control agents and for the reestablishment of vegetation on disturbed sites.

Three species of alder were chosen for study in this research: European black alder (_Alnus glutinosa_ (L.) Gaertn.), hazel alder (_Alnus serrulata_ (Ait.) Willd.), and seaside alder (_Alnus maritima_ Muhl. ex Nutt.). Alders were chosen in particular because of available data from research done previously and because they appear to be the most prolific nodulators of the actinorhizal plants. The black alder is also a potential source of timber (as in Europe). The two other species, hazel and seaside alder, happen to be native to Oklahoma, which could prove advantageous in the search for drought tolerant varieties.

European black alder is a medium-sized tree, or occasionally, a large shrub introduced from Europe that is cultivated primarily in eastern Canada and the United States (Appendix B, Figure 1). It also now reproduces naturally in low, wet, or swampy sites. This is a fast growing alder that may reach up to 30m.

Hazel alder is primarily a shrub of the eastern United States, ranging from Maine to northern Florida, and as far west as Oklahoma (Appendix B, Figure 2). It grows on moist lowlands such as swamps and along ponds and streams. Hazel alder must grow in full sun and will die out if shaded by taller trees. It can attain heights of 10m, usually with multiple and somewhat spindly trunks. Dense pure thickets can be found, but this alder more often grows in association with river birch, swamp cottonwood, willows, and red maple.

Seaside alder, a shrub or small tree, occurs in the coastal regions of southern Delaware and southeastern Maryland and then reappears in southcentral Oklahoma (Appendix B, Figure 3). No other tree species within the United States has a greater distance between its disjunct areas (72). Seaside alder grows in wet soils, primarily along streams and ponds at lower elevations. It can attain heights of 10m with straight, narrow trunks. It does form dense, pure stands in swampy areas. Common trees growing in association with this alder include red maple, sweetgum, river birch, and willow.

## Procedure

Clonal lines developed from the three species of alder were used in this experiment. The parental stock of seaside alder and hazel alder was produced from native Oklahoma stands (Appendix B, Figure 4). Hazel alder was collected in central McCurtain County just west of Beavers Bend State Park along one of several small creeks found in the area. The source for seaside alder was found in central Johnston County, northeast of Tishomingo, along the Blue River. Hardwood cuttings were taken from each stand in March, 1980, and transported to the forestry greenhouse in Stillwater in an iced cooler. There, they were planted in labeled six inch plastic pots containing a 2:1 mix of perlite and vermiculite. The pots were then placed in a small mist chamber giving intermittent mist at 15 seconds per 15 minute period. Once rooting and new growth was evident, they were moved out of the mist chamber onto the greenhouse benches and allowed to put on more growth. Eventually, the healthily growing specimens were transplanted to one gallon size plastic pots containing a 2:1 blend of Jiffy Mix, a commercial sphagnum moss potting medium, and Oil-Dry, a high capacity clay absorbent. The latter component was added to increase drainage.

Stock for black alder was established through international seed sources. An extensive compilation by Iowa State University under the auspices of the NC-99 regional tree improvement program provided a wealth of Alnus seed sources to choose from. Selection was limited to sites lying within the same general latitude as Oklahoma (37' 15" N). The seed obtained was germinated in Spencer-Lamaire root trainers containing a blend of Jiffy Mix and Oil-Dry under intermittent mist of 12 seconds per 15 minutes. Healthy seedlings were eventually transplanted to larger pots of the same medium and placed out in the greenhouse. During propagation of all cuttings and seeds, a liquid solution of Peters 20-20-20 plus micronutrients fertilizer was applied twice a week to offset leaching caused by the mist system and extensive watering of the rapidly growing potted plants. In addition, stock plants were sprayed every three weeks with Plictran (4.5 g/11.36 liters water) to control mites.

Clonal lines were developed by taking selected 15cm apical cuttings from specific parental stock, exposing them 10 seconds to a liquid solution of 8000ppm indole-3-butyric acid (Appendix A, Table I), and dusting the cut ends with a 5% concentration of benomyl. The treated cuttings were inserted into a large propagation bed made up of a six to eight inch depth of moist vermiculite and perlite (1:2) under a mist system of 12 seconds per 10 minute period. The bed was maintained at an ambient temperature of 25-27 degrees Celsius by a buried heating cable. This series of treatments and environmental conditions proved to increase the survivability and rooting of the cuttings, as compared to earlier cuttings which did not receive these specific treatments. Approximately six weeks were required for the establishment of roots after which the clones were weaned from the mist and later transplanted into six inch plastic pots containing Jiffy Mix and Oil-Dry. This process of establishing unnodulated rooted cuttings from a single original cutting or single seed was repeated numerous times in order to generate sufficient clonal stock to undertake the experiment.

Prior to the initiation of the experiment, 18 uniformly, unnodulated rooted cuttings of a single isogenetic clone were selected for each species. The clone of black alder used was recorded in the Iowa State list as AG8022; this seed source was Astaneh-Ashrafieh, Iran. The clone for hazel alder was simply designated 'As' and the one for seaside alder 'Am.' Ten grams of Osmocoat 19-6-12, a time release fertilizer, and 0.10g Timek, a systemic insecticide, were added to each pot. The pots were numbered and color coded for ease in identifying species and treatments and placed in a growth chamber

(Percival PGW132 Special) for 10 days of acclimation. One week prior to the initiation of the experiment, the pots were enclosed in plastic bags (tied around the lower stem) to prevent evaporation. During this preparatory time all clones were maintained at well watered conditions.

The experiment was designed as a series of three periods each 10 days in length for a total of 30 days. Periods were initiated on day 0, 10, and 20 when differing phases of the watering regime were introduced for the moderate and severe moisture levels. The water levels for each treatment were determined as a percentage of each species' initial water requirement. Two things became evident when working with potted plants in the greenhouse and in an earlier experiment which provided the rationale for this procedure. First, it became apparent that each species had its own unique water requirement. Because demands for water were not similar, the amount of water needed to maintain a given treatment level would necessarily need to differ for each species. Second, the water levels could not be reliably based upon the field capacity of the potting material alone. The demands of fast growing species which in nature prefer moist locations would quickly outgrow the limited capacity of a soil mix contained in a six inch pot. Therefore, field capacity in the strictest sense of the word proved impractical as a basis for establishing treatment levels, and a more tailored approach to the individual requirements of each species seemed to be in order. In the greenhouse, clones of each species, established as previously described, were placed in six inch pots. The pots were then sealed in plastic bags to insure that the water lost was due solely to plant evapotranspiration. In the preliminary experiment, plants were monitored daily in the growth chamber, following a 10 day acclimation period, to determine the amount of water used and to quantify how much water was required to return each plant to a well-watered (control) condition. Within a week's time it became evident that these measurements were fairly consistent and that an average initial water requirement could be assigned to each species based on such a series of measurements. Inducing water stress was then achieved by depriving each species of a fixed percentage of their initial total water requirement. For European black alder this initial amount was 135ml, for hazel alder it was 116ml, and for seaside alder it was 76ml. The plants at the onset of the experiment were quite small in stature compared to their size at the termination; this explains the rather small quantities of water initially needed. The moderately-stressed level for period one was established at 75% of the initial requirement and the severely-stressed level at 50%. Those amounts were then reduced for period two by one-third of one-quarter of the total initial amount, setting the moderate level at 66.93% and the severe level at 41.76%. For period three, the reduction was two-thirds of one-quarter, dropping the moderate level to 58.69% and the severe level to 33.51%. It was the desire of the investigator to induce stress gradually so as to reflect what would more likely happen in the field. Also, growth would prove a factor in magnifying the actual reductions and it was desired that the plants would, for the most part, survive the duration of the experiment so that viable measurements of stress could be taken. With all this in mind, the three watering regimes, by period, were as follows:

Control: maintained at well watered condition, daily, for all three species.

Moderate Stress:

black alder 102ml, 92ml, 80ml
hazel alder 58ml, 52ml, 46ml
seaside alder 88ml, 78ml, 68ml
added daily on days 0-9, 10-19, 20-29, respectively.

Severe Stress:

black alder 68ml, 58ml, 46ml
hazel alder 38ml, 32ml, 26ml
seaside alder 58ml, 50ml, 40ml
added daily on days 0-9, 10-19, 20-29, respectively.

During the experiment, ambient air temperature was 22.22-26.67 Celsius for the 16 hour days and 15.56-18.89 Celsius for the eight hour nights. Relative humidity, measured with a hygrothermograph, varied from 62 to 70%. The light quantum density at the level of the plants averaged 716 microEinstein per meter squared per second as measured with a quantum sensor (Model LI-85 Quantum Radiometer with quantum sensor, Li-Cor, Lincoln, Nebraska).

Starting on October 1, 1981, stomatal resistance, leaf area, and height were measured on alternate days for one month to expedite a comparative study of plant tolerance and growth under duress. First, due to sensitivity to environmental fluctuations, such as light intensity, temperature, and air currents, abaxial stomatal resistance at the tip and to the side of the midrib of the fourth expanded leaf of the main stem was measured with a calibrated stomatal diffusion porometer (Model LI-700 Transient Porometer, Li-Cor, Lincoln, Nebraska). Second, height was determined to the nearest 0.5cm by resting a meter stick on the lip of the pot and measuring the length of of the designated main stem to the base of the apical meristem. Third, a point on the main stem just below the first two or three mature leaves was flagged and the area of the leaves above this point was measured with a transparent dot grid based on square centimeters.

Leaf and height measurements were taken from the main stem, since most of the growth would be located in that region on such young plants. In order to standardize the measurement of stomatal resistance, the fourth fully developed leaf from the apex (LPA=4) was selected for all three species. Measurement of stomatal resistance was consistently taken from the underside of the leaf, because the bulk of the stomates are located on this side through which most of the plant's transpiration takes place. Height was measured from the lip of the pot to the base of the apex due to the difficulty in accurately determining the point of stem-root interface. It was felt that monitoring leaf area growth from a specific part of the main stem could be used as an indicator of clonal drought sensitivity, rather than measurement of total plant leaf area. In addition, because of the abundant number of leaves on each plant, the time required to calculate the total leaf area by dot grid at each measurement day would have been excessive. Therefore, at the start of the experiment, an initial point just below the first two mature leaves was marked with a twist tie, and all subsequent measurements of leaf expansion were made above this point. The choice of parameters and their employment as described above are accepted as standard methods used in drought stress research. The reliability and sensitivity of these particular parameters has been attested to in reports by Morrow and Slayter (54, 55), Kanemasu et al. (42), Hsiao (41), Zahner (86), and recently by Kelliher and Tauer (44). The articles by Hsiao and Zahner (41, 86) contain particularly informative discussions on the value of various growth parameters as indices of drought sensitivity.

The statistical design was a split plot in time with three species, three treatments, and six replications for a total of 54 experimental units. With three sets of measurements (stomatal resistance, leaf area, and height) taken from three species undergoing three different treatments, a total of 27 variables were studied. Analysis, in general, focused upon the blocks, stress regimes, alder species, and the species-regimes interactions. A preliminary analysis consisted of an examination of mean variation via original, absolute (day "x" minus day 1), and relative (day "x" minus day 1, divided by day 1) data analysis. Several important and significant responses were recognized but the massiveness and complexity of the analysis prevented a clear and unquestionable interpretation of established trends. To remedy this problem, the variation of the overall mean for each period was studied through a combination of regression and analysis of variance. A regression analysis by species and treatment was performed to evaluate the variation in response per day for each of the variables. Results from this analysis suggested that linear relationships could be applied to each period. These lines were plotted against the original data and were found to be highly compatible. The means for each period were then derived from these projected linear relationships for statistical comparisons made via an analysis of variance. The projected mean values, which correspond by period with the midpoint on each regression line, were consistent with the mean values for each period of the original data. The result of this analysis was a more clear establishment of response trends allowing for more confident interpretation of the data.

## Results

### Stomatal Resistance

Of the three species tested, black alder control plants were higher in stomatal resistance than either hazel alder or seaside alder (Appendix A, Tables II, III, and IV). Black alder control plants maintained resistance values from 1.57 to 14.76 s/cm (seconds per centimeter), while hazel alder values were from 1.22 to 6.59 s/cm and seaside alder values ranged from 1.88 to 5.68 s/cm (Appendix A, Table XI). It was interesting to note that there were no significant differences (P=.05) between the three species while under well-watered conditions. A notable difference was anticipated due to the fact that both hazel and seaside alder were derived from native stands in the southernmost regions of Oklahoma and would be more likely adapted to periodic drought conditions. Conversely, black alder was procured from

European sources located near the latitude that parallels Oklahoma's northern border. Minor differences were apparent, but overall the control groups of the three species were remarkably similar in degree and pattern of response. This similarity in response suggests that it may not be feasible to screen Alnus clones for differences in drought tolerance by simply maintaining them under the same soil moisture regime. Plotted regression lines can be compared in Appendix B, Figures 6, 8, and 10.

An anomaly arose in the resistance values of the two remaining stress regimes for black alder (Appendix A, Tables II, III, and IV). By period two there were significant differences between the moderately-stressed and control levels, and between the severely-stressed and control levels. There were no significant differences between the moderate and severe levels. Moderately-stressed black alder gave a span of stomatal resistances from 1.52 to 134.39 s/cm, while the severely-stressed plants had a lower range of 1.71 to 66.20 s/cm (Appendix A, Table XI). This discrepancy was apparently revealing black alder's native preference for moist sites. Even at the outset of the experiment they proved to be significantly sensitive to the initial reduction in water under the moderately-stressed treatment. Apparently, the black alders were more capable of handling the stresses met under the moderate regime. Stomatal resistance climbed steeply from the beginning and peaked within the third period after which severe wilt and leaf burn caused a small decline (Appendix B, Figure 6). The black alders of the severely-stressed treatment, conversely, were strained beyond their normal tolerance capabilities early in the experiment. In order to survive, they underwent structural changes in limb and leaf which caused the difference in stomatal resistance readings. Apical dominance was apparently lost, producing three lesser branches growing away from each other and bending to a horizontal angle. The leaves had become small, extremely thin and fragile, and yellow-green in color. It was not clear whether this was an adaptive measure taken by the plants or a fluke of the experiment, for the moderately-stressed plants never took on this change, even near the extreme resistance values found.

The moderately and severely-stressed hazel alders had lower stomatal resistance values than the corresponding black alders (Appendix A, Tables II, III, and IV: and Appendix B, Figures 6 and 8). By period two both treatments were statistically significant from the controls but not from each other. Hazel alder was also significantly different from black alder in its response to the moderate and severe regimes. Resistances for the moderately-stressed plants were from 1.56 to 37.39 s/cm, while resistances of the severely-stressed plants ranged from 1.79 to 40.03 s/cm (Appendix A, Table XI). Mean resistance values increased sharply with increasing moisture stress, but not as steeply as black alder, suggesting a difference in drought tolerance between the two species.

The moderately and severely-stressed seaside alders maintained the lowest stomatal resistance values of the three species (Appendix A, Tables II, II, and IV; and Appendix B, Figures 6, 8, and 10). By period two, the severe treatment was significantly different from the controls but not from the moderate level, similar to the hazel alders. The two regimes were also significantly different from the black alders in their stress responses. Resistances for the moderately-stressed seaside alders were from 1.81 to 23.19 s/cm, while resistances of the severely-stressed plants ranged from 1.20 to 55.79 s/cm (Appendix A, Table XI). Resistances of the stressed seaside alders were seen to increase with increasing water stress, but to a lesser degree than the other two species. This suggests not only that there was a difference in drought tolerance from the other two species, that is worth further investigation, but also that seaside alder could survive well in drier environs, which is in conflict with the general literature.

## Leaf Area

Based on the regression data, leaf area expansion was greatest for black alder and seaside alder under the control regime (Appendix A, Tables V, VI, and VII). For hazel alder the control group of plants remained the least productive of the three regimes until the third period where they finally began outgrowing the severely-stressed group. Wide species differences were evident from the strong statistical significance maintained throughout the experiment. Black alder held the largest increase of 694.67 square centimeters, characteristic of its broad, thin, planar leaves. Seaside alder ranked second in leaf area expansion with 565.17 square centimeters, while hazel alder rated third with a 291.50 square centimeter increase (Appendix B, Figures 11, 14, and 17).

Black alder exhibited extreme sensitivity to moisture reduction in the moderately and severely-stressed treatments (Appendix B, Figures 11, 14, and 17). Leaf expansion was reduced to 374.17 square centimeters in the moderately-stressed group, nearly half of what the control plants were able to produce. Inhibition of leaf expansion appeared to be the primary cause. New leaves were continually being produced but as the stresses progressed into period three, the leaves took on a pronounced quilted or waffled appearance. There was little leaf senescence and abscission taking place. The severely-stressed plants had undergone what appeared to be structural changes in response to the sharp reduction in moisture. The total expansion of this group was 366.80 square centimeters consisting of comparatively smaller, flat, extremely thin and fragile, yellow-green colored leaves. Abscissions became more prevalent towards the end of period three with death of the plant following.

Moderately and severely-stressed hazel alder produced the least amount of leaf area expansion with 263.17 square centimeters being grown by the moderately-stressed plants and 211.00 square centimeters by the severely-stressed plants (Appendix B, Figures 11, 14, and 17). Due to an unexpected burst of growth early in the experiment, the moderate and severe means were disproportionate in comparison to the controls. This may have been a sign that hazel alder grows better in drier soils, but unlikely. Throughout the propagation of hazel alder it was learned that it traumatized rather easily requiring a long period of time for recovery. It is believed that this was the cause of the results obtained. Cessation of new leaf production and abscission were the primary methods of leaf area reduction in response to water stress. Leaves generally were smaller and darker in color with some quilting evident on occasion.

Seaside alder produced the most relative leaf growth (Appendix B, Figures 12, 15, and 18) of all three species while under moisture stress, and ranked second in total leaf area expansion with 461.00 square centimeters for the moderately-stressed and 342.50 square centimeters for the severely-stressed groups (Appendix B, Figures 11, 14, and 17). Inhibition of leaf area expansion appeared to be primarily due to a reduction in size. There was no observable change in number of leaves as in the hazel alders or in structure as in the black alders except for a slight cupping toward the underside of the leaf. Seaside alder even surpassed black alder in leaf area expansion while under duress in period three. This would suggest that seaside alder leaves were able to maintain a more favorable water balance for growth than the leaves of the other two species despite the drought stress conditions imposed.

## Height

Total height growth for the control plants was greatest for black alder with an increase of 24.75 cm (Appendix B, Figure 20). Seaside alder control plants held the next largest increase in height with 20.25 cm while hazel alder grew 16.00 cm (Appendix B, Figures 23 and 26). This ranking was maintained throughout the experiment at all three treatment levels. It was thought that hazel alder would likely attain a similar stature to seaside alder due to a higher relative increase in growth, but this never materialized. Strongly significant differences were found in the growth habits of each species.

Black alder was again revealed to be highly sensitive to even initial moisture deprivation (Appendix A, Tables VIII, IX, and X). The moderately and severely-stressed plants grew significantly less from the controls but not from each other. As a matter of fact, both levels responded with remarkably similar measurements. The moderately-stressed group grew an average of 15.33 cm while the severely-stressed plants nearly matched that with 15.10 cm. Their manner of growth was very different though. The moderately-stressed plants retained the more characteristic single, slender upright trunk with minimal branching. The severely-stressed plants, on the other hand, appeared to have lost their apical dominance and had produced three main trunks growing away from each other in a triangular symmetry. This was thought to be a structural response to the severe moisture deprivation experienced by the plants. Both groups exhibited reduced internodal distances.

Moderately and severely-stressed hazel alder revealed height growth patterns similar to black alder, but yet significantly different in amount of growth (Appendix A, Tables VIII, IX, and X). Neither of the watering regimes were significantly different in growth but instead exhibited unusually similar measurements. The moderately-stressed plants grew 9.83 cm while the severely-stressed plants grew 9.00 cm (Appendix B, Figurres 20, 23, and 26). Reduction of internodal spacing was observed in both regimes but more so in the severe treatment. Several of the severely-stressed plants practically ceased expansion between nodes and instead stacked the nodes one upon another creating very tightly clustered bunches of foliage.

Relative height growth of seaside alder rivalled and even surpassed black alder (Appendix B, Figures 21, 24, and 27). Seaside alder stood the second tallest of the three species in total increase in size. The moderately-stressed plants increased 15.75 cm with the severely-stressed plants growing 9.88 cm. There were significant differences in growth between the control plants and severely-stressed plants of seaside alder and between the severely-stressed seasides and the corresponding black alders. The moderately-stressed seaside alders had nearly attained the same height of the black alder counterparts. Visual observation revealed that stress did reduce the expansion between internodes but not to the degree found in the hazel alder.

# CHAPTER IV

## REVIEW AND DISCUSSION

The ultimate goal of this project involves the investigation of the effects of water-stress on dinitrogen fixation by Alnus species as a function of the xerotolerance of the host and endophyte. The desirability of improved xerotolerance in Alnus is being stressed more and more by silviculturalists and plant scientists alike (33, 59). Yet little is known of the water requirements of alders as a species nor the intraspecific variation in response to moisture stress under controlled conditions.

The development of alders of improved xerotolerance required, as the first order of study, the establishment of a base line of response to water stress for unnodulated, but fertilized, species of Alnus. Clonal materials of seaside alder and hazel alder was collected from native stands in Oklahoma. Clones of European black alder were propagated from internationally collected seed. Following rooting, clones were potted, placed within a controlled environment chamber, and subjected to three levels of progressive water stress covering a one month period. Stomatal resistance, leaf area expansion, and height increase were measured for indications of drought sensitivity.

There has been considerable interest in the use of diffusive resistance as a measurement of drought sensitivity for the past decade. The primary route of vapor exchange from plant back to environment is via leaf stomata. Consequently, variations in stomatal aperture would, in turn, markedly affect stomatal resistance revealing the degree of sensitivity a plant has for droughty conditions. In this study stomatal resistance proved to be a highly sensitive indicator of water stress. Stomatal resistance was also much quicker to respond to stressful conditions than the growth indicators used.

Leaf expansion proved to be a sensitive indicator of water stress (86). It also was found to reveal the individual nature of each specie's growth response to stress. Where stomatal resistance depicted the degree of stress the plant was suffering, a study of leaf expansion revealed how the plant translated this suffering into modified growth characteristics. This is an extremely important trait, for an investigator would want to know whether a tree undergoes modification of its leaves and retains vigorous growth or resorts to massive abscission or undesirable structural degeneration in an attempt to survive. Such information would be essential to making decisions on selection, breeding, and commercialization.

Height growth was found to be less sensitive to water stress than leaf expansion or stomatal resistance. Generally, there was poor discrimination among the different levels of treatment except at extreme ranges. Its usefulness was mostly limited to establishing the suspected differences in growth habits of each species. It was helpful in detailing individual response to moisture stress only when included with leaf expansion and stomatal resistance. Together, these three measurements proved quite valuable in the comparative study of clonal response to water stress.

Black alder was discovered to be acutely sensitive to water deprivation. Even initial reductions in moisture had a profound influence on its character as compared to the control plants. With a sharp relative increase (as calculated from the regression data) in stomatal resistance of 349.92% for the moderately-stressed plants there was a 35.07% averaged decrease in potential leaf area and a 32.05% drop in height. This and the peculiar structural degeneration that occurred with the severely-stressed black alders suggests that they are unsuitable for growth in arid and semi-arid environments in their present state. Perhaps McVean (52) was correct in stating that black alder had little or no practical stomatal control. It would appear that they had little control over growth when subjected to stress. What modifications did occur seemed to result from sheer lack of materials, namely water, rather than any adaptive measure. The tendency toward rapid growth may have proved detrimental to this species for survival in drier climates.

Hazel alder proved to be an analytical enigma which could have been due to some error on the part of the investigator as much as any eccentricity of the species itself. The moderately and severely-stressed groups revealed a general increase in leaf area over the controls until the later part of the third period where the severely-stressed plants dropped below the controls. There was also much less reduction in height growth than observed in the other species. This may have been due to a misjudgment by the investigator. The hazel alders were found to be more difficult to work with during propagation. They traumatized easily and grew much slower or not at all as a result. It is quite possible that many of the ramets selected may not have had well developed root systems. This would account for the irregularities witnessed. Also, it is quite possible that the investigator may have misjudged the approximate initial water requirement of this species and thereby overcalculated the water levels needed for the experiment. The fact that the moderately-stressed plants outgrew the control plants suggests that either there was a disproportionate number of poorly rooted plants in the controls or they were suppressed by an overly saturated soil. Alders in general prefer a moist but well-drained soil. The pots being enclosed in plastic bags could hardly be considered as well drained if the pots were being over watered to begin with. There was very little runoff evident during the experiment. Hazel alder showed a marked averaged increase (as calculated from the regression data) of 102.28% and 172.59% for stomatal resistance in the moderately and severely-stressed plants, respectively. Leaf area expansion exhibited a general increase of 29.77% and 27.82%, while height growth decreased 12.72% and 14.39% for the respective stress groups. Hazel alder was the smallest in stature of the three species. While growth was irregular it did reveal a stronger adaptive trend to stress than black alder. Further investigation of this species may prove fruitful. It is known to extend over a wide region of the United States. Hardier varieties may be discovered which could be tested and refined for some useful purpose.

Seaside alder surpassed hazel and black alder in stomatal control and growth under duress. With an averaged increase (as calculated from the regression data) of 104.06% and 117.45% in stomatal resistance for the moderately and severely-stressed groups, seaside alder maintained the lowest overall resistance values of the three species. Also, when a new reduction in the water levels was introduced at each stress level, there followed an initial flare-up in resistance values which then tended to settle back to a somewhat lower value. This suggested that seaside alder may be able to facilitate internal adjustments more efficiently than the others. Potential leaf expansion decreased 10.14% and 29.23%, respectively, for the moderately and severely-stressed plants, while height growth decreased 23.99% and 34.65%, respectively. Seaside alder was able to attain and surpass even black alder in leaf area while under stress. Height grew comparably to black alder as early as period two. Seaside alder was apparently better able to tolerate stress through greater control of its stomatal activity and its capacity for internal adjustment of its tissues. Reduction in relative height was more than leaf area, perhaps suggesting that the leaves could better maintain their water balance. Such maintenance minimized damage to the plant, allowing it to modify its growth while balancing its needs between maximum production and optimum survival. As a result, less growth was lost to stress.

It should be noted that the data recorded herein is descriptive only of one clone from each species studied under the particular conditions prescribed. Though the environment was designed to simulate a generalized Oklahoma climate, caution is advised in their interpretation and application to field studies or other populations and related species.

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# APPENDIXES

# APPENDIX A

# TABLES

## TABLE I

## IBA ROOTING HORMONE SOLUTION
(8000ppm, 2% ETOH)

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Materials:

IBA powder (Sigma I1875)
2N NaOH
2N HCl
100% ETOH
Distilled Water

Directions:

1. Mix 0.8g IBA in 4ml of 2N NaOH. Stir.
2. Add 2ml of 100% ETOH. IBA should be in solution.
3. Dilute to 92ml by slowly adding distilled water.
4. Slowly adjust pH to ca. 6.3 with 2N HCl.
   If IBA precipitates, back titrate with 2N NaOH until precipitate disappears. Then, very slowly readjust pH to ca. 6.3 with 2N HCl.
5. Make final dilution to 100ml with distilled water.

---

Note:

Keep refrigerated and protected from light to prevent oxidation of hormone. Use immediately. This formula has a very short shelf life.

## TABLE II

## STOMATAL RESISTANCE MEANS FOR PERIOD ONE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 3.59* | 6.06 | 4.75 | 4.80 |
| Hazel Alder | 3.20 | 3.93 | 4.28 | 3.80 |
| Seaside Alder | 3.19 | 4.12 | 3.83 | 3.72 |
| Average | 3.33 | 4.70 | 4.29 | |

*$LSD_{.05}$= 1.79 For comparing species at the same stress levels or stress levels of the same species within a given period.

N= 6

Unit of measurement for stomatal resistance is s/cm (seconds per centimeter).

## TABLE III

## STOMATAL RESISTANCE MEANS FOR PERIOD TWO

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 4.24* | 11.55 | 11.06 | 8.95 |
| Hazel Alder | 3.59 | 6.26 | 8.01 | 5.95 |
| Seaside Alder | 3.77 | 5.95 | 6.83 | 5.52 |
| Average | 3.87 | 7.92 | 8.63 | |

$^*LSD_{.05} = 2.58$ For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for stomatal resistance is s/cm (seconds per centimeter).

## TABLE IV

## STOMATAL RESISTANCE MEANS FOR PERIOD THREE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 3.82<br>(0.2429)* | 34.76<br>(8.4668) | 21.93<br>(4.6085) | 20.17 |
| Hazel Alder | 4.18<br>(0.2429) | 12.02<br>(2.2591) | 17.64<br>(2.2591) | 11.28 |
| Seaside Alder | 3.88<br>(0.2429) | 12.03<br>(2.2591) | 12.89<br>(2.2591) | 9.60 |
| Average | 3.96 | 19.60 | 17.49 | |

*The number in parentheses below a given value is the standard error of that specific value. Due to the size of variance for some of these values, it was not possible to compile all of them into a single group for analysis. Therefore , a single LSD value could not be calculated for the entire group.

N= 6

Unit of measurement for stomatal resistance is s/cm (seconds per centimeter).

## TABLE V

## LEAF AREA MEANS FOR PERIOD ONE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 129* | 98 | 97 | 108 |
| Hazel Alder | 49 | 73 | 66 | 63 |
| Seaside Alder | 80 | 73 | 66 | 73 |
| Average | 86 | 81 | 76 | |

*LSD $_{.05}$= 32 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for leaf area is cm$^2$.

## TABLE VI

## LEAF AREA MEANS FOR PERIOD TWO

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 362* | 234 | 242 | 279 |
| Hazel Alder | 113 | 148 | 155 | 139 |
| Seaside Alder | 254 | 236 | 182 | 224 |
| Average | 243 | 206 | 193 | |

*$LSD_{.05}$= 90 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for leaf area is $cm^2$.

## TABLE VII

## LEAF AREA MEANS FOR PERIOD THREE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 695* | 378 | 363 | 479 |
| Hazel Alder | 240 | 263 | 212 | 238 |
| Seaside Alder | 491 | 420 | 289 | 400 |
| Average | 475 | 354 | 288 | |

*LSD $_{.05}$= 179 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for leaf area is cm$^2$.

# TABLE VIII

## HEIGHT MEANS FOR PERIOD ONE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 28.00* | 21.00 | 22.00 | 23.50 |
| Hazel Alder | 9.50 | 11.00 | 12.00 | 10.50 |
| Seaside Alder | 18.00 | 13.50 | 12.50 | 14.50 |
| Average | 18.50 | 15.00 | 15.50 | |

*$LSD_{.05}$= 7.00 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for height is the cm.

## TABLE IX

### HEIGHT MEANS FOR PERIOD TWO

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 42.00* | 28.00 | 28.50 | 33.00 |
| Hazel Alder | 15.50 | 15.00 | 15.50 | 15.00 |
| Seaside Alder | 26.00 | 20.00 | 17.00 | 21.00 |
| Average | 28.00 | 21.00 | 20.00 | |

*$LSD_{.05}$= 9.00 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for height is the cm.

## TABLE X

## HEIGHT MEANS FOR PERIOD THREE

| Species | Control | Moderate Stress | Severe Stress | Average |
|---|---|---|---|---|
| European Black Alder | 52.00* | 33.00 | 33.00 | 39.00 |
| Hazel Alder | 21.50 | 18.50 | 18.00 | 19.00 |
| Seaside Alder | 33.00 | 25.00 | 20.00 | 26.00 |
| Average | 35.50 | 25.50 | 23.50 | |

*$LSD_{.05}$= 10.00 For comparing species at the same stress level or stress levels of the same species within a given period.

N= 6

Unit of measurement for height is the cm.

# TABLE XI

## MAXIMUM-MINIMUM VALUES FROM THE ORIGINAL DATA

| SPECIES | STRESS* | PERIOD | STOMATAL RESISTANCE (s/cm) |  | LEAF AREA ($cm^2$) |  | HEIGHT (cm) |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  | LOWER | UPPER | LOWER | UPPER | LOWER | UPPER |
| European Black Alder | C | 1 | 1.59 | 11.61 | 22 | 299 | 7.5 | 49.0 |
|  | C | 2 | 1.58 | 14.76 | 66 | 733 | 11.0 | 58.0 |
|  | C | 3 | 2.05 | 7.53 | 187 | 1158 | 6.0 | 73.0 |
|  | M | 1 | 1.52 | 47.55 | 34 | 206 | 11.0 | 28.5 |
|  | M | 2 | 3.31 | 42.88 | 94 | 470 | 19.5 | 38.0 |
|  | M | 3 | 3.93 | 134.39 | 136 | 688 | 24.5 | 39.5 |
|  | S | 1 | 1.71 | 13.05 | 27 | 274 | 9.0 | 41.5 |
|  | S | 2 | 3.75 | 41.51 | 104 | 352 | 16.0 | 44.0 |
|  | S | 3 | 0.00 | 66.19 | 226 | 600 | 23.0 | 44.5 |
| Hazel Alder | C | 1 | 1.22 | 6.56 | 20 | 125 | 1.0 | 23.5 |
|  | C | 2 | 2.03 | 5.17 | 37 | 233 | 6.5 | 33.5 |
|  | C | 3 | 2.48 | 6.59 | 76 | 521 | 10.5 | 40.0 |
|  | M | 1 | 1.56 | 10.55 | 33 | 130 | 1.0 | 21.5 |
|  | M | 2 | 1.79 | 11.64 | 58 | 313 | 2.0 | 27.0 |
|  | M | 3 | 3.22 | 37.39 | 70 | 536 | 5.0 | 28.0 |
|  | S | 1 | 1.79 | 10.94 | 27 | 139 | 6.0 | 18.5 |
|  | S | 2 | 4.68 | 21.29 | 64 | 299 | 11.0 | 20.5 |
|  | S | 3 | 0.00 | 40.03 | 53 | 463 | 14.0 | 21.0 |
| Seaside Alder | C | 1 | 1.88 | 5.55 | 25 | 144 | 11.0 | 27.0 |
|  | C | 2 | 2.12 | 5.53 | 103 | 571 | 15.5 | 40.0 |
|  | C | 3 | 2.41 | 5.68 | 226 | 891 | 22.0 | 49.0 |
|  | M | 1 | 1.81 | 8.43 | 17 | 150 | 5.0 | 22.5 |
|  | M | 2 | 3.09 | 14.15 | 132 | 383 | 12.0 | 27.0 |
|  | M | 3 | 6.68 | 23.19 | 292 | 606 | 18.5 | 30.5 |
|  | S | 1 | 1.20 | 7.63 | 18 | 141 | 7.0 | 21.0 |
|  | S | 2 | 4.09 | 12.62 | 89 | 356 | 10.5 | 25.0 |
|  | S | 3 | 4.78 | 55.79 | 95 | 514 | 15.0 | 27.0 |

*Stress levels are identified as follows: C=control groups, M=moderate stress, S=severe stress.

# APPENDIX B

## FIGURES

Figure 1. General Distribution of European Black Alder in the United States

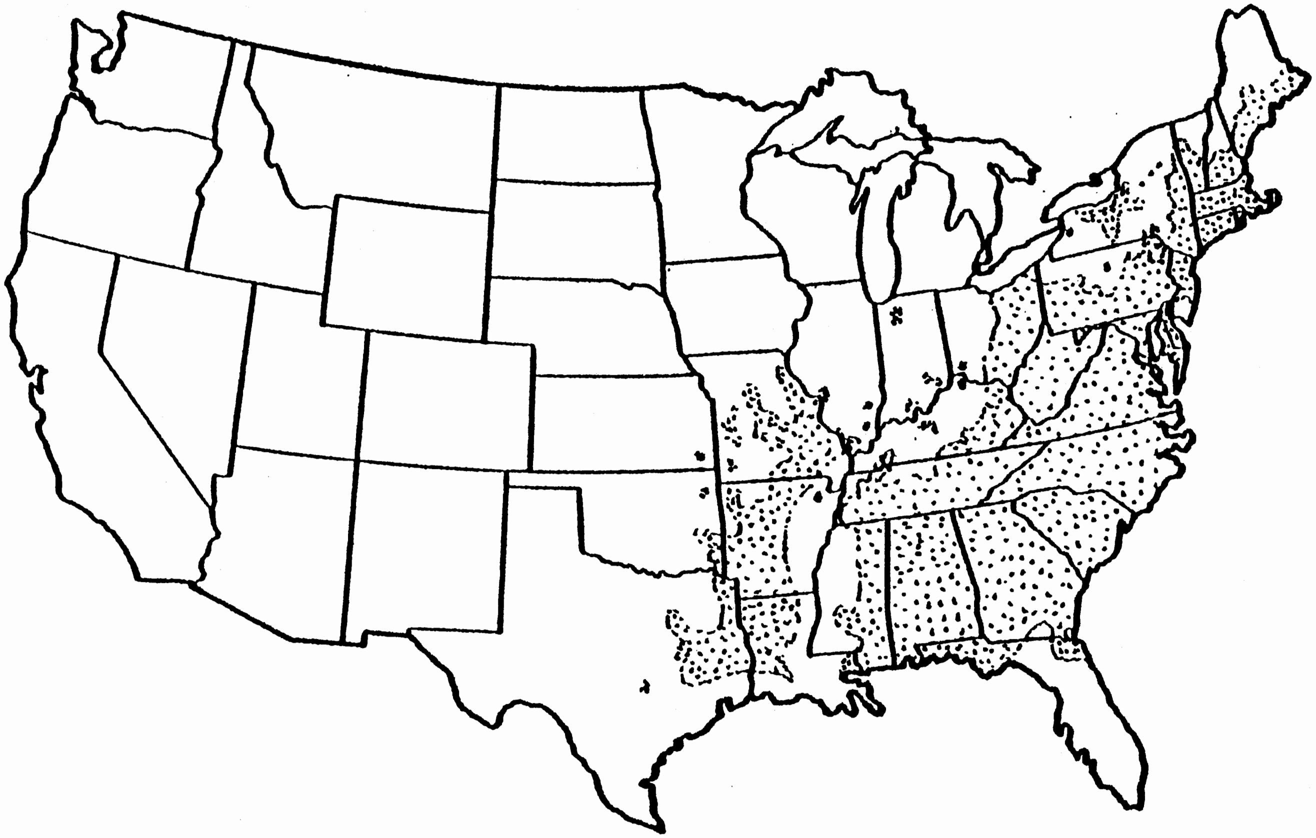

Figure 2. Distribution of Hazel Alder in the United States

Figure 3. Distribution of Seaside Alder in the United States

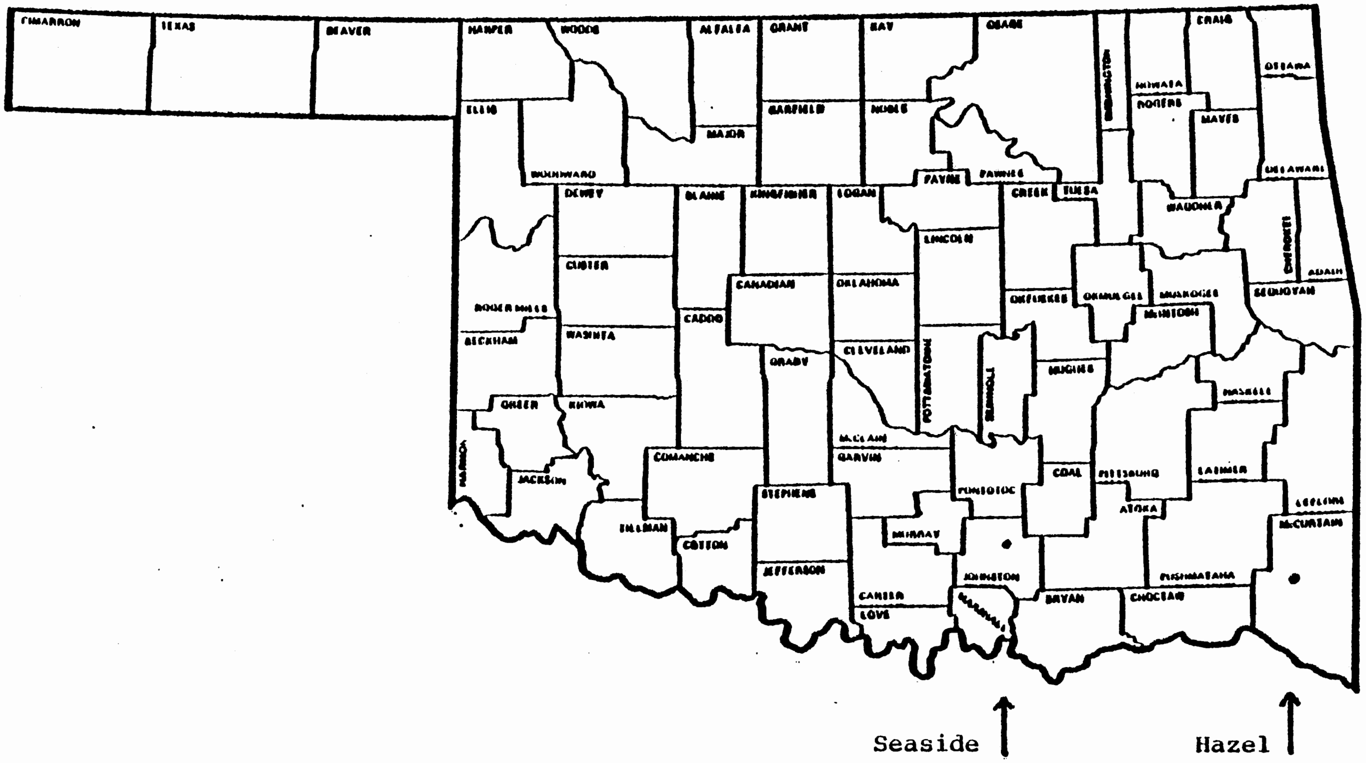

Figure 4. Collection Sites for Seaside and Hazel Alder

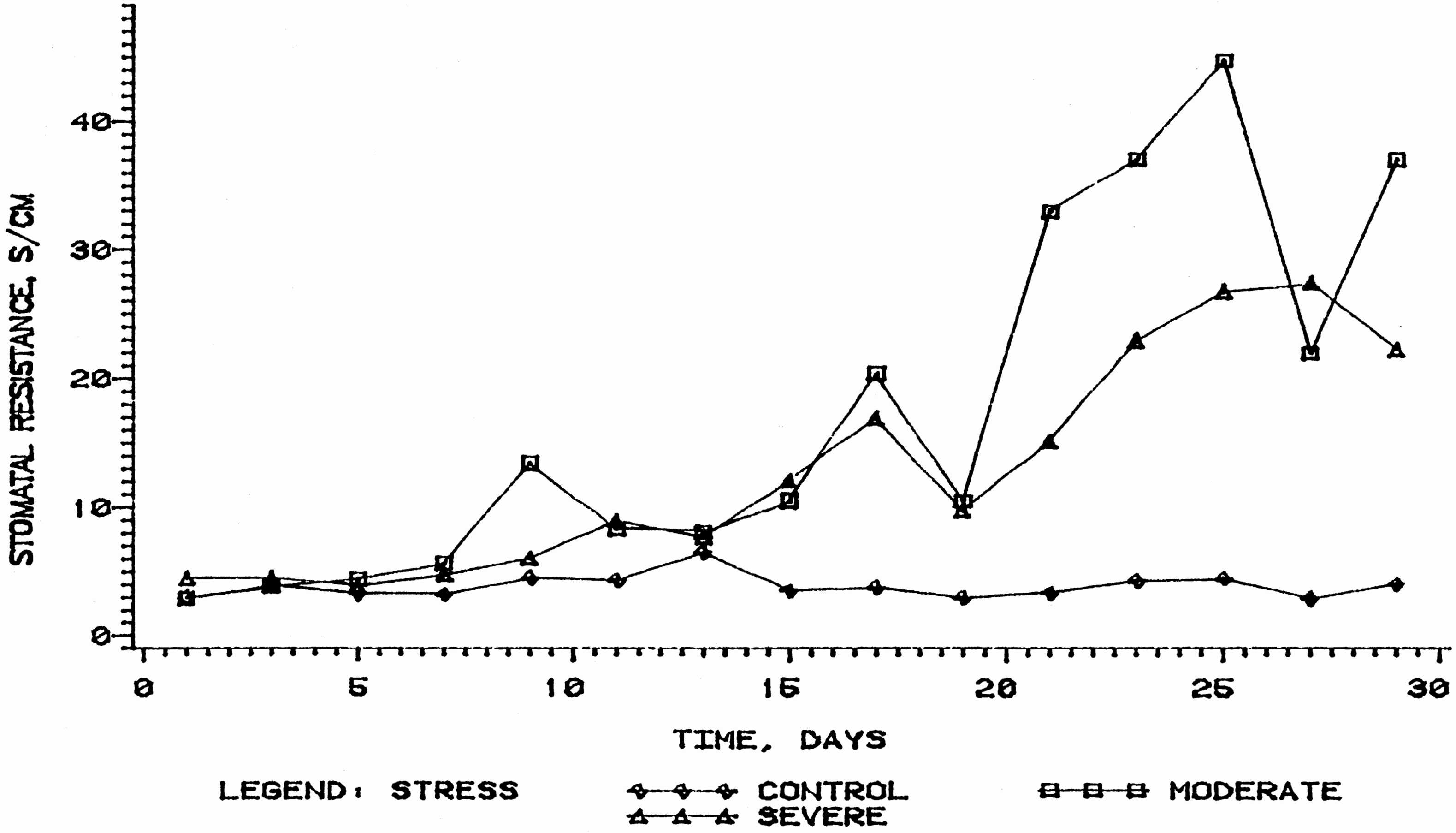

Figure 5. Stomatal Resistance of European Black Alder Under Three Watering Regimes as Plotted From the Original Data

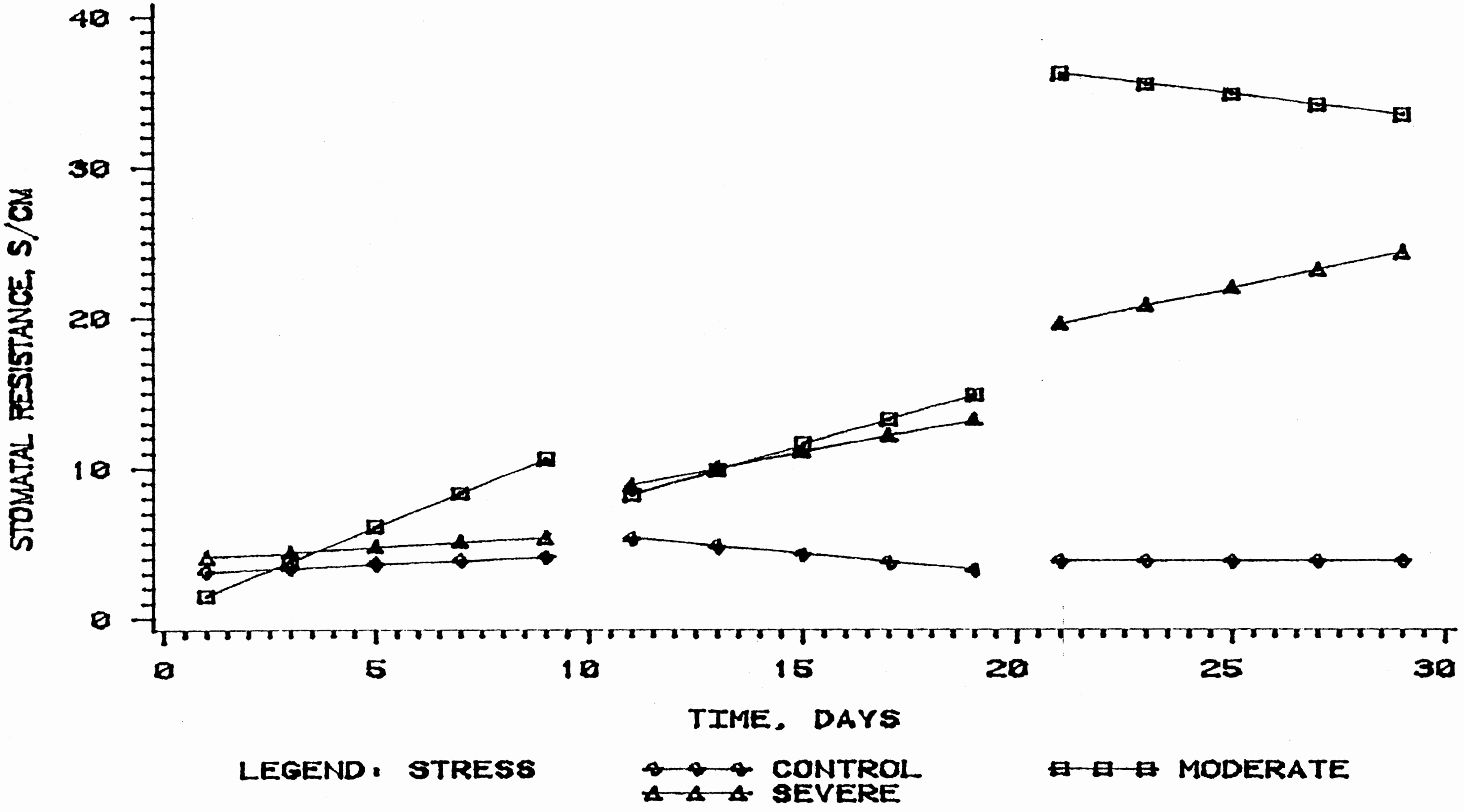

Figure 6. Plot of Regression Lines by Period for Stomatal Resistance of European Black Alder Under Three Watering Regimes

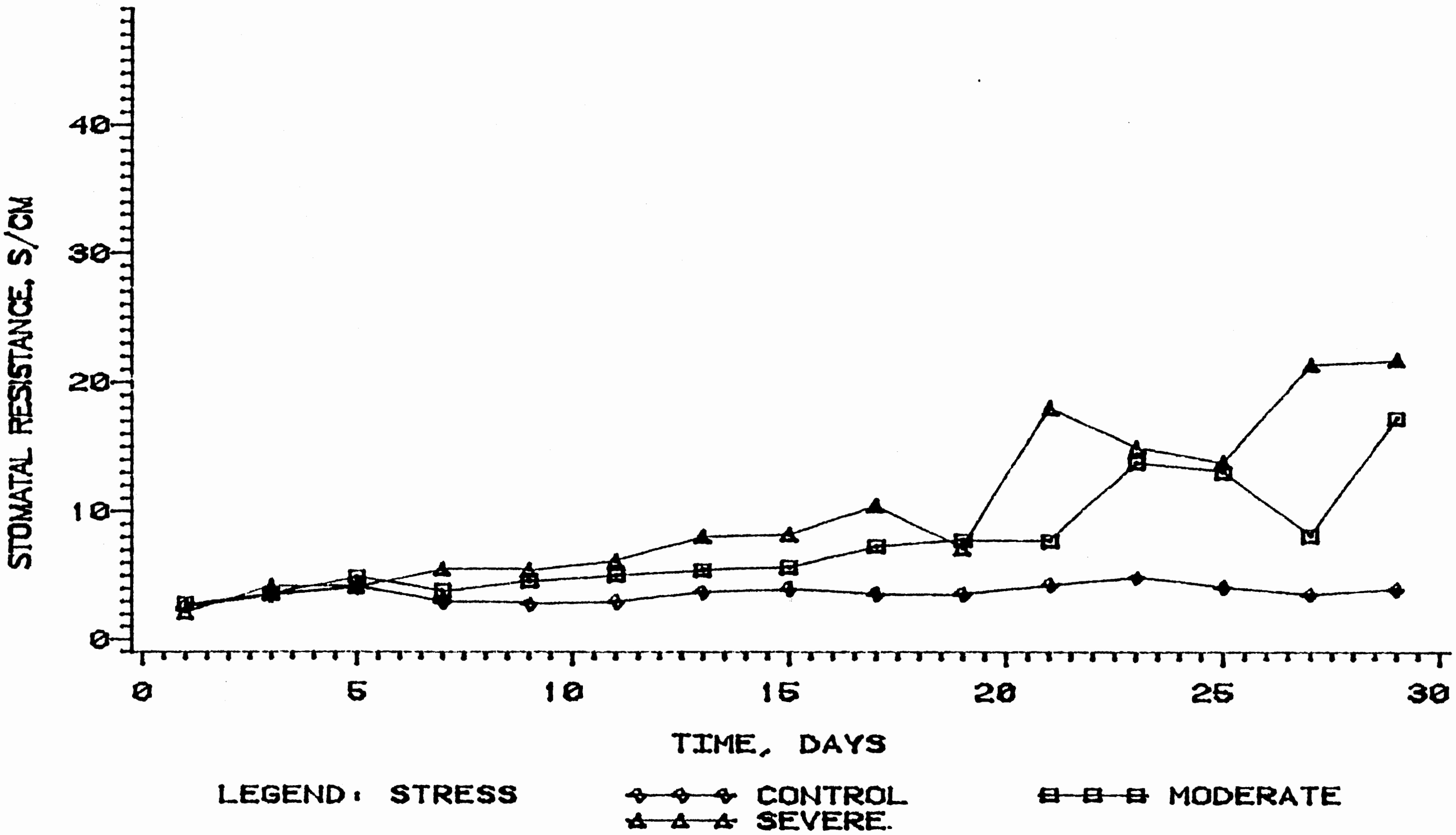

Figure 7. Stomatal Resistance of Hazel Alder Under Three Watering Regimes as Plotted From the Original Data

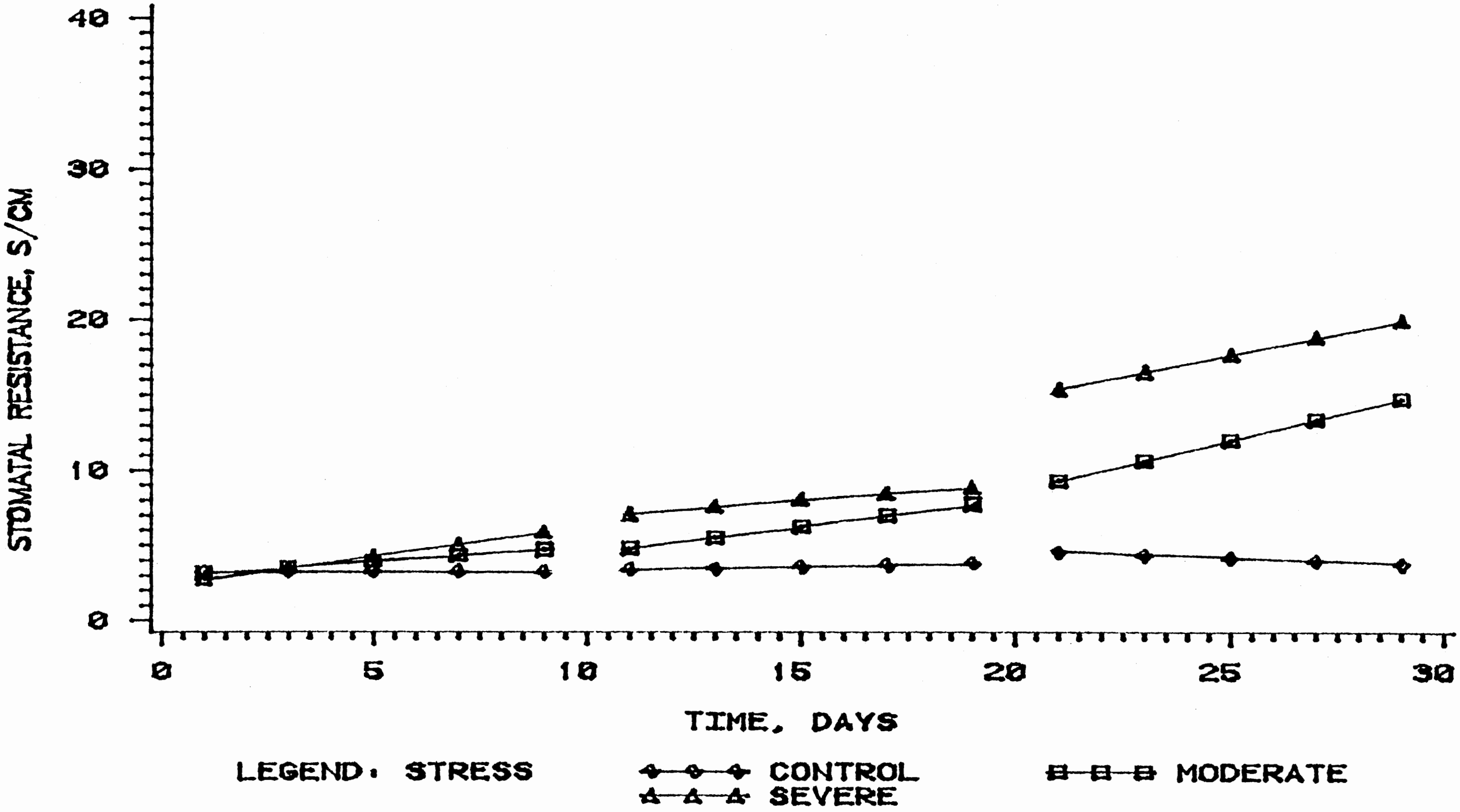

Figure 8. Plot of Regression Lines by Period for Stomatal Resistance of Hazel Alder Under Three Watering Regimes

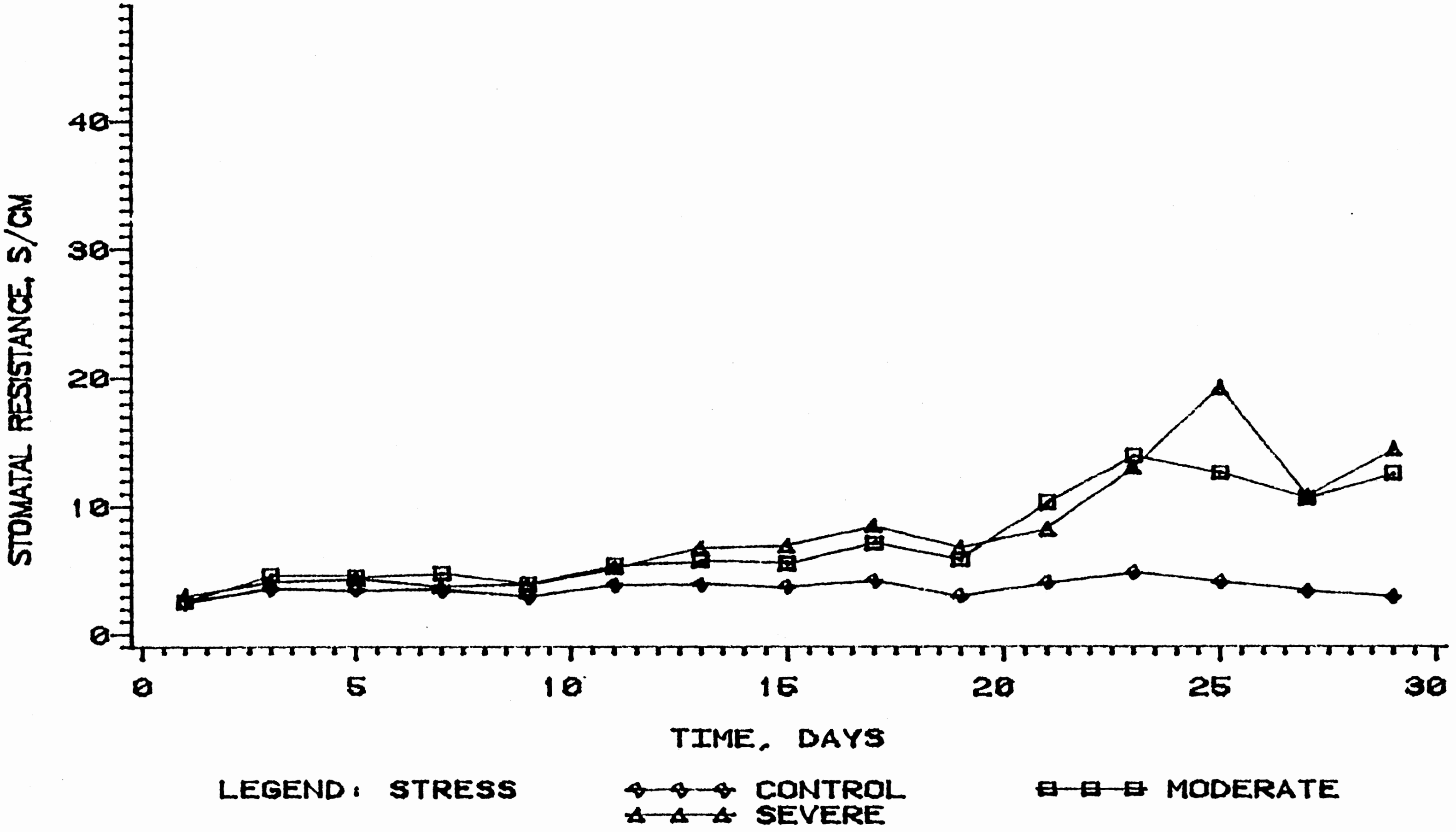

Figure 9. Stomatal Resistance of Seaside Alder Under Three Watering Regimes as Plotted From the Original Data

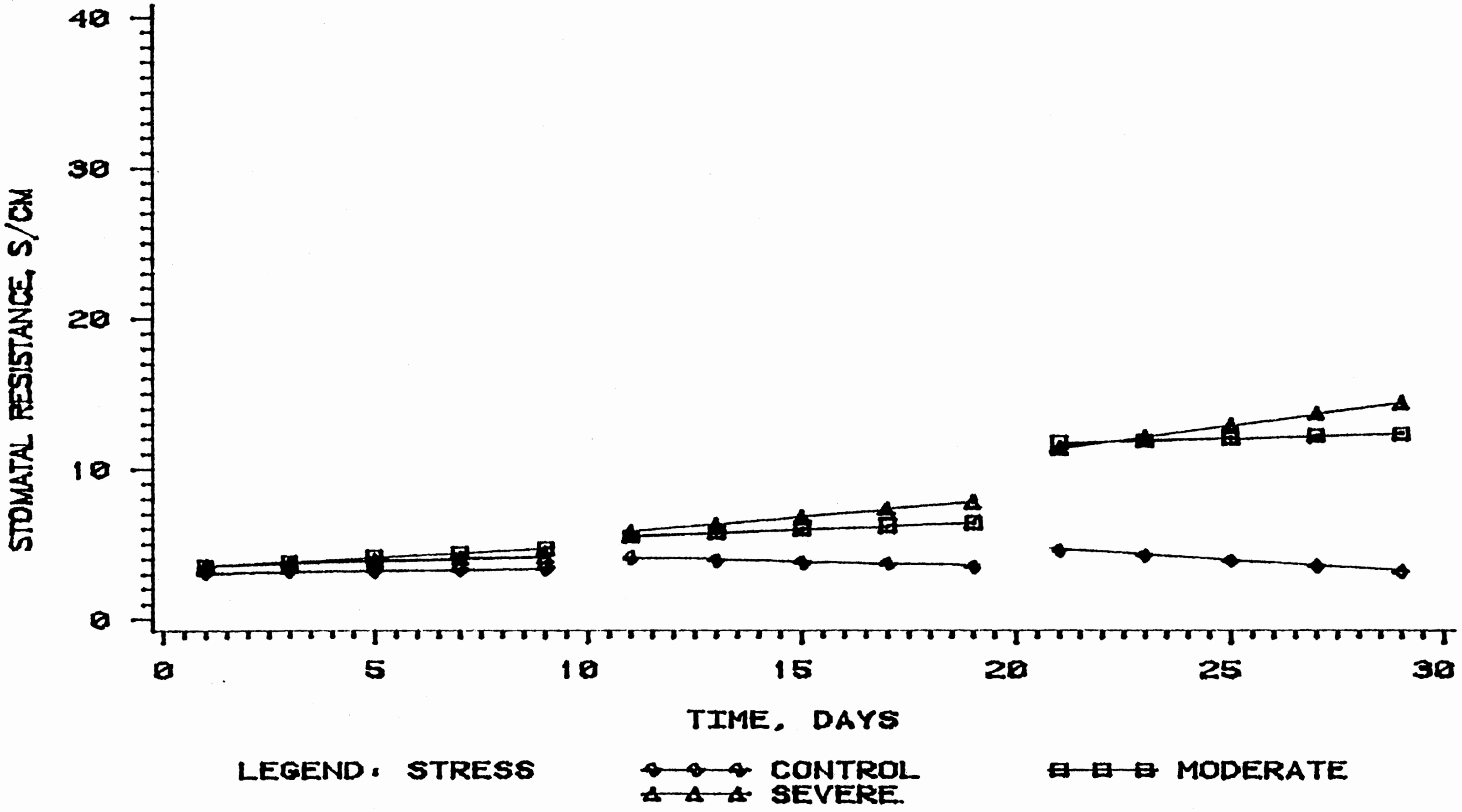

Figure 10. Plot of Regression Lines by Period for Stomatal Resistance of Seaside Alder Under Three Watering Regimes

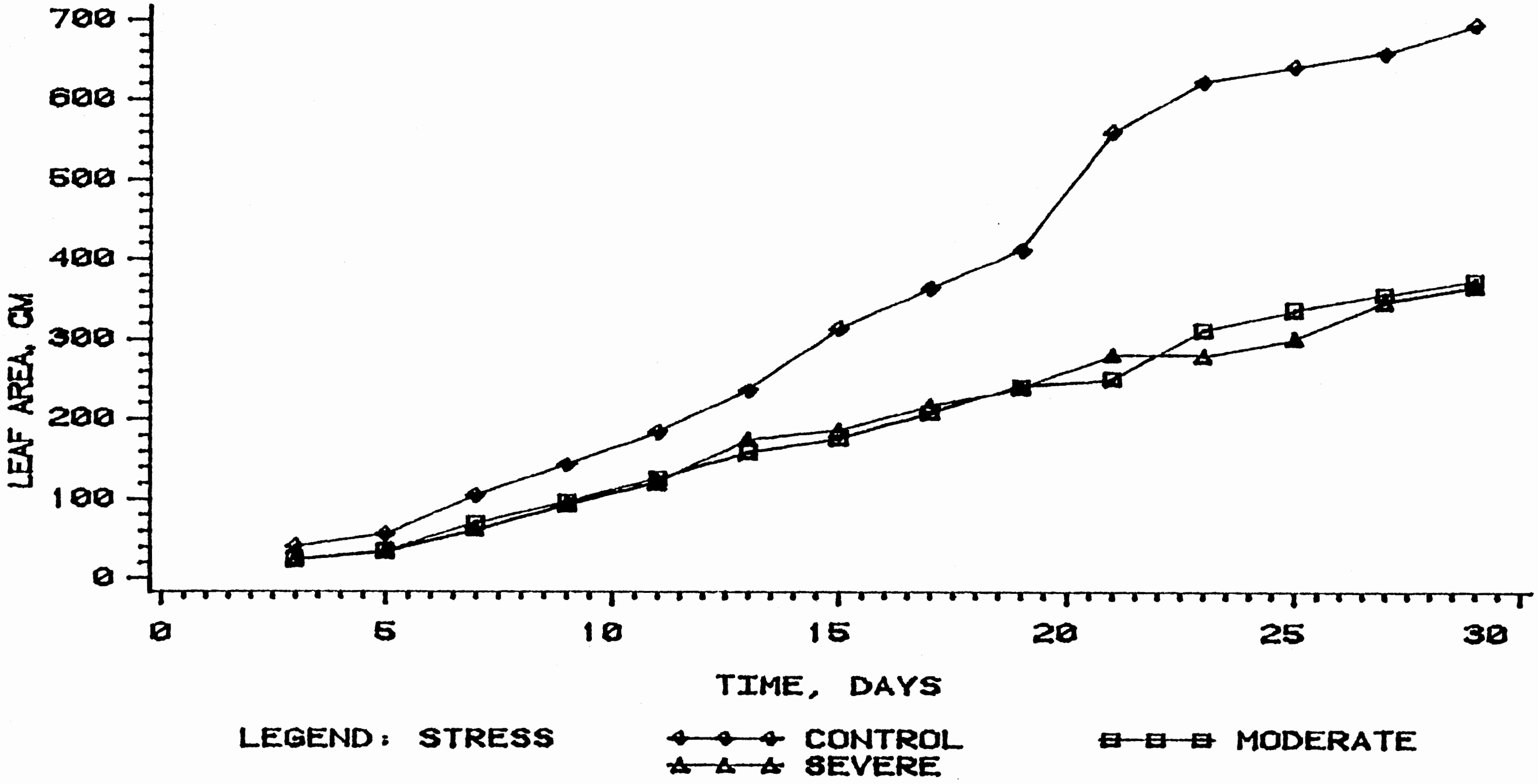

Figure 11. Absolute Change in Leaf Area of European Black Alder Under Three Watering Regimes

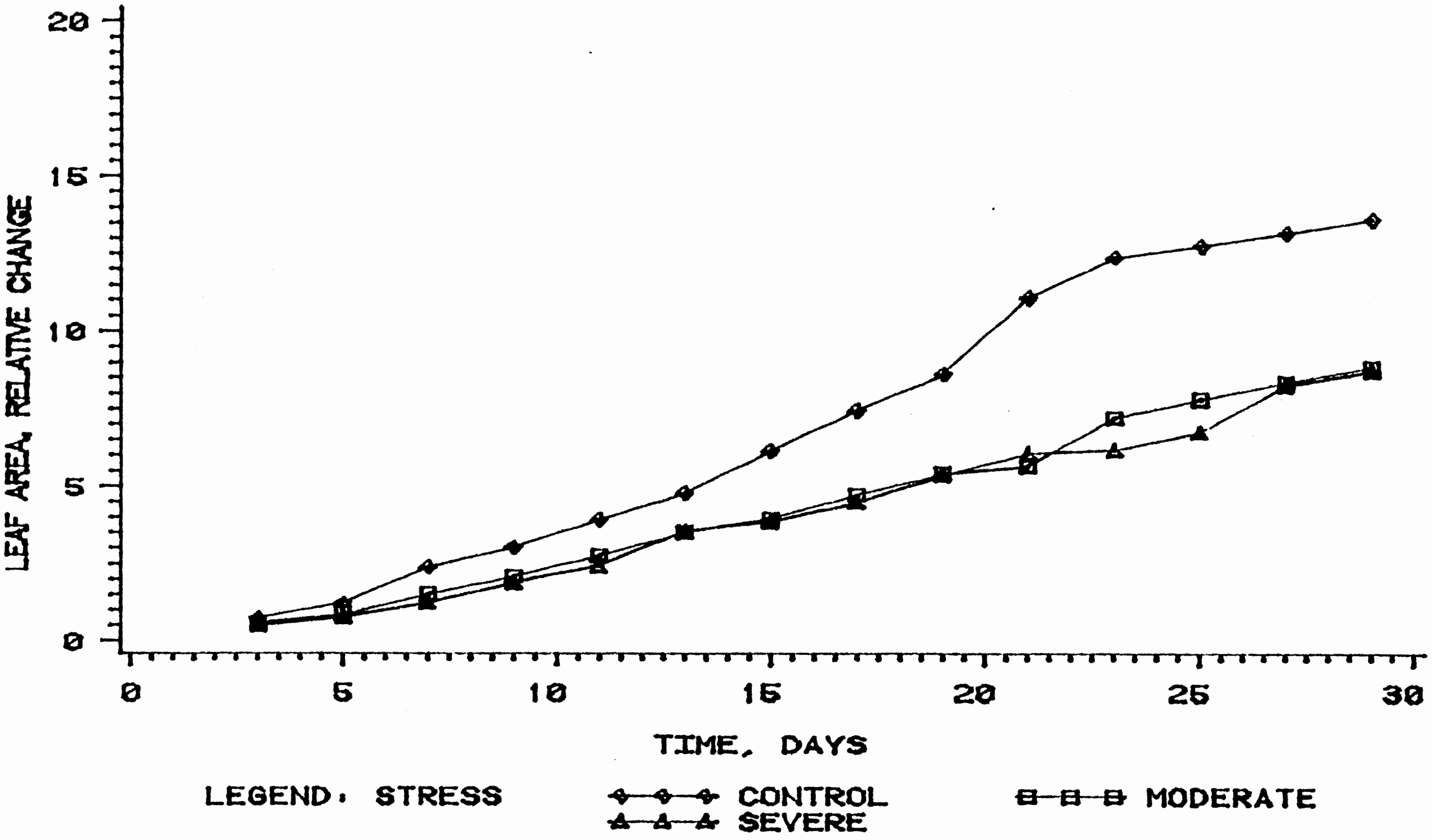

Figure 12. Relative Change in Leaf Area of European Black Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

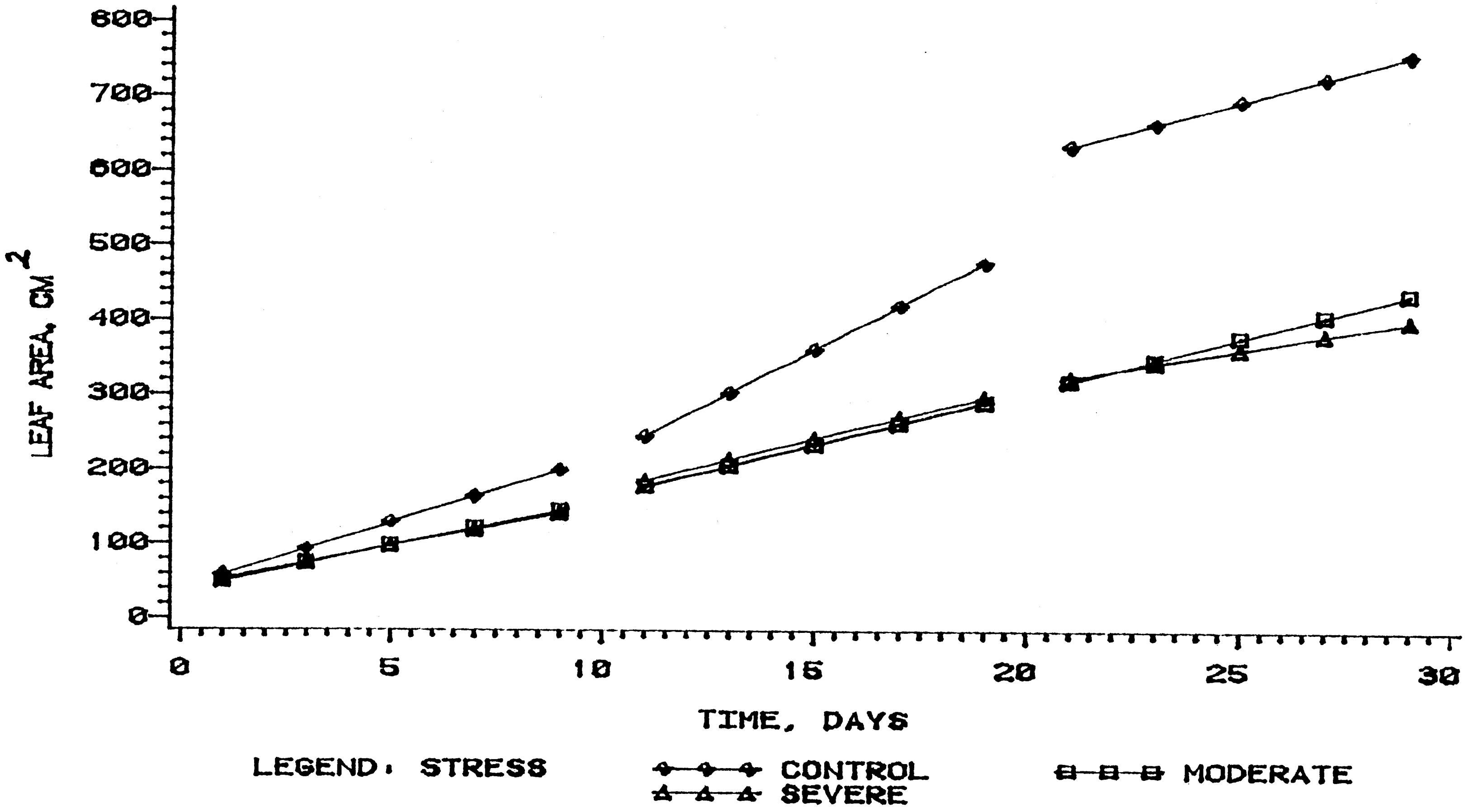

Figure 13. Plot of Regression Lines by Period for Leaf Area of European Black Alder Under Three Watering Regimes

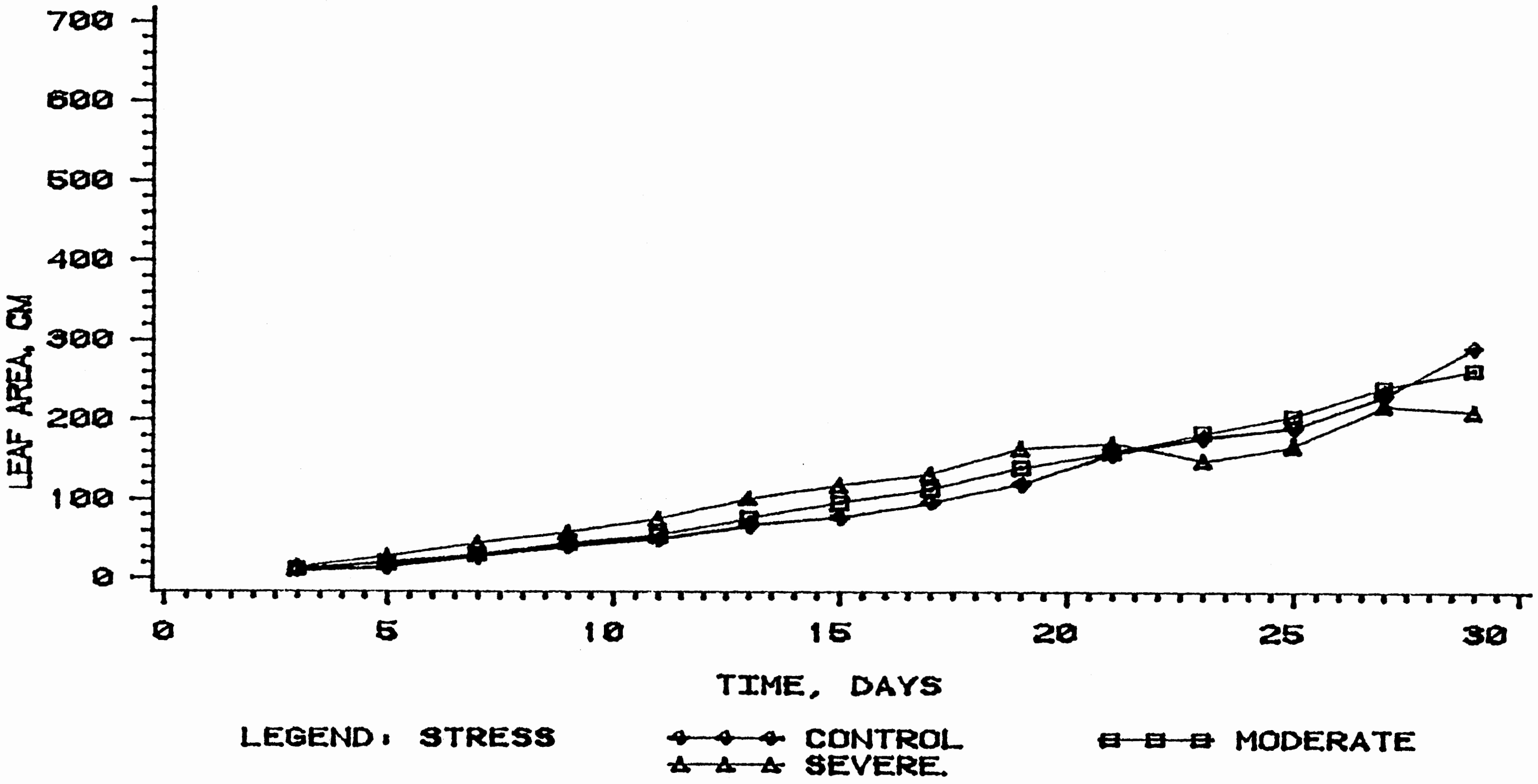

Figure 14. Absolute Change in Leaf Area of Hazel Alder Under Three Watering Regimes

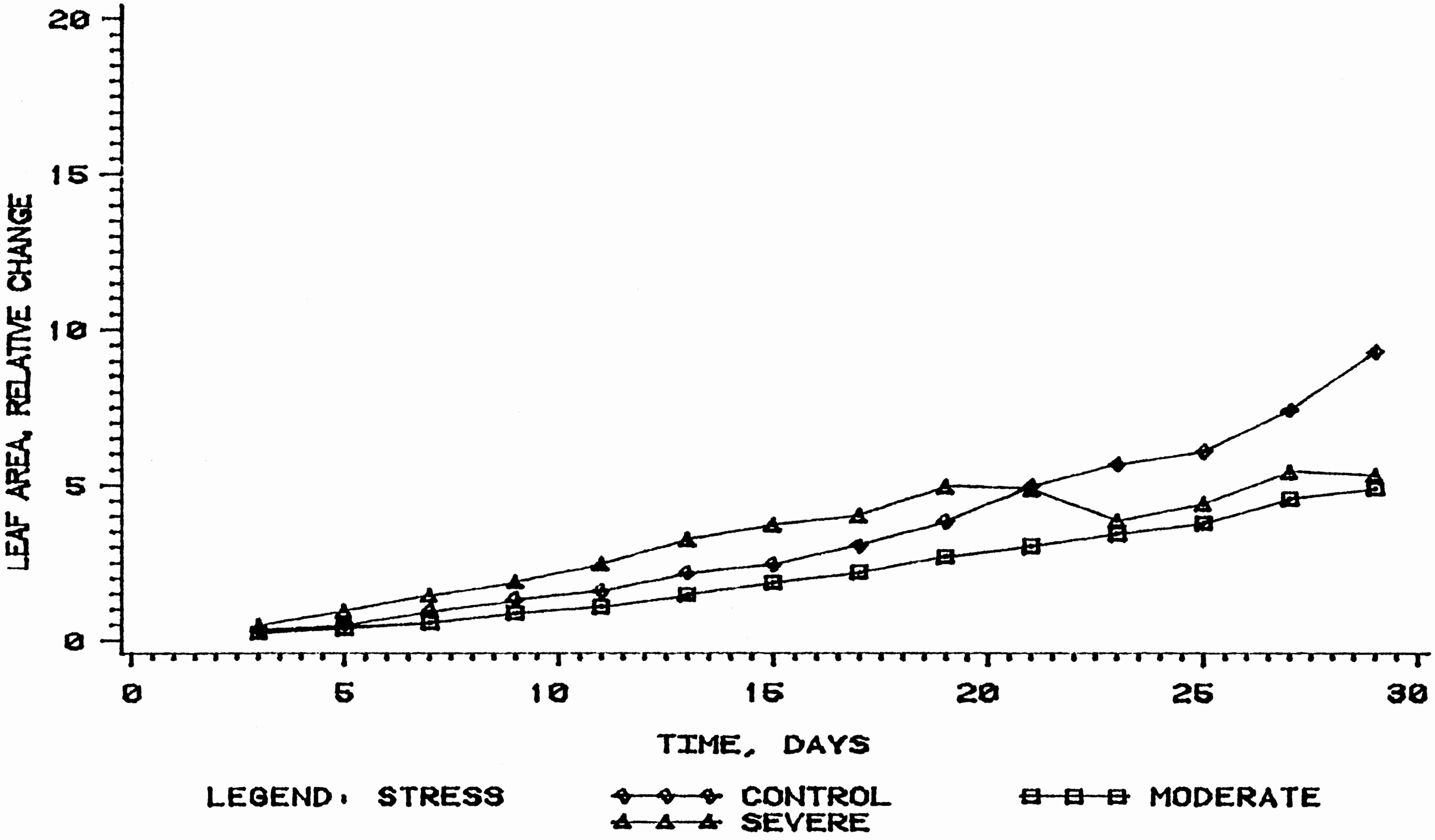

Figure 15. Relative Change in Leaf Area of Hazel Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

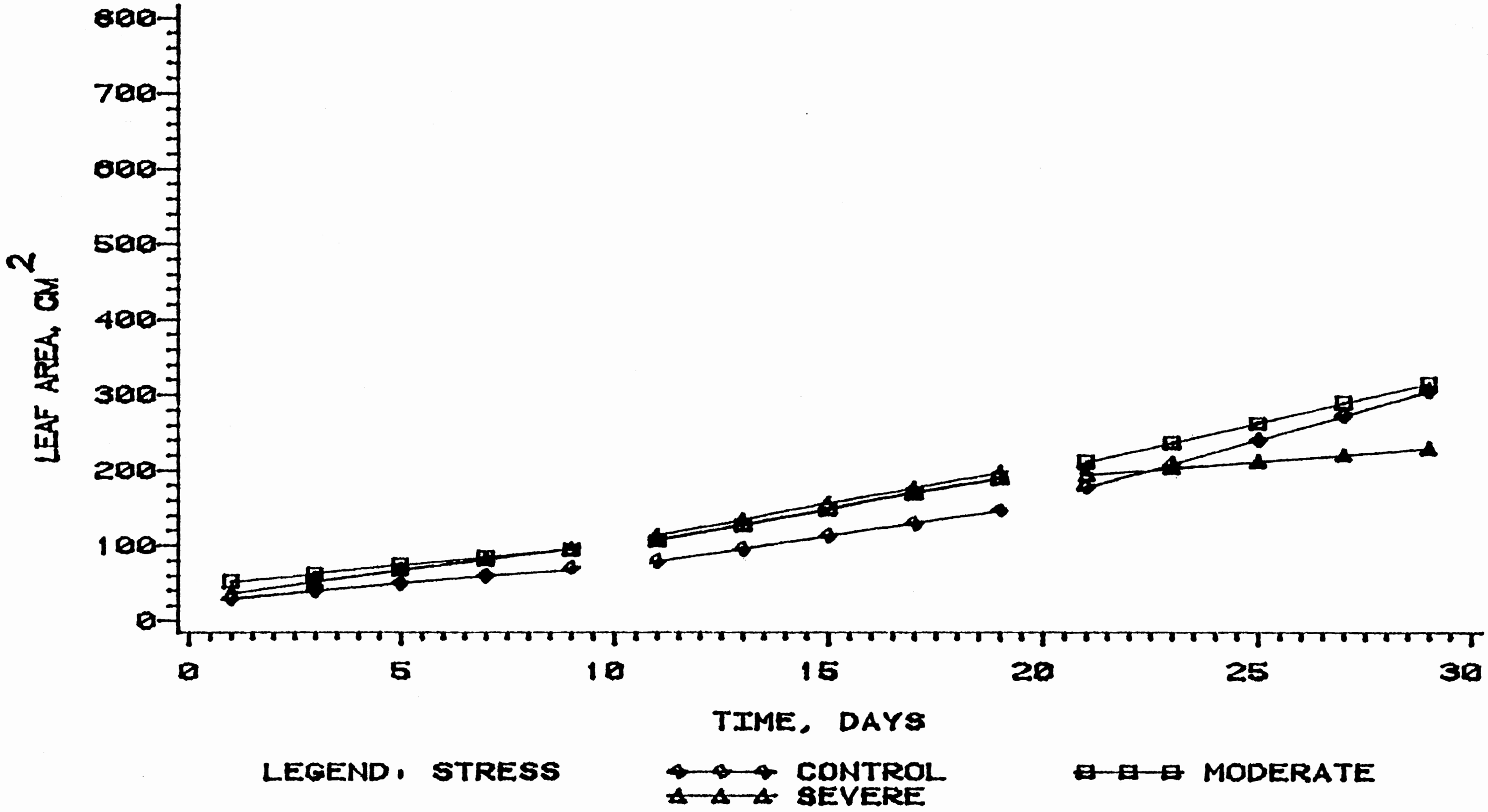

Figure 16. Plot of Regression Lines by Period for Leaf Area of Hazel Alder Under Three Watering Regimes

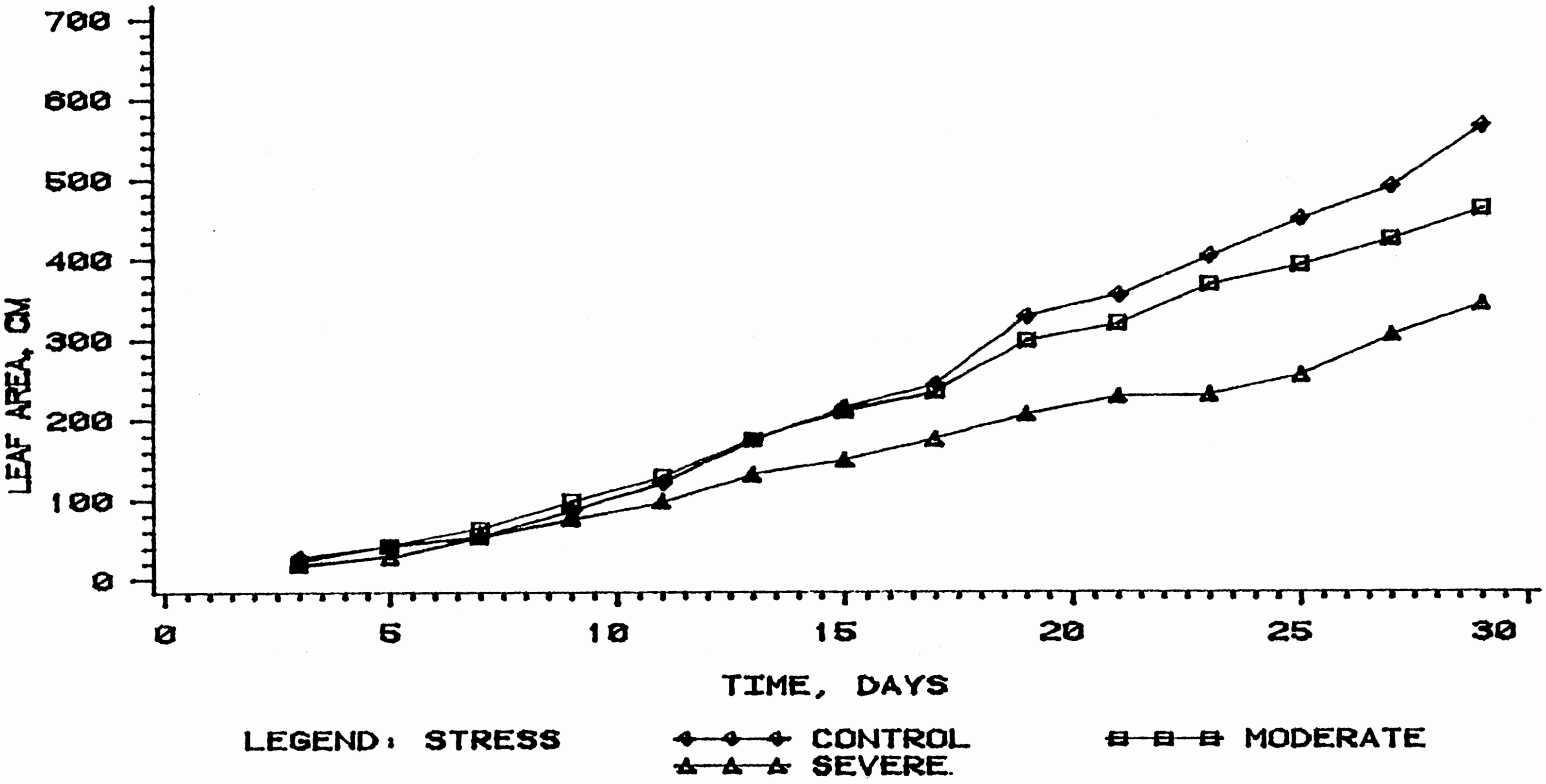

Figure 17. Absolute Change in Leaf Area of Seaside Alder Under Three Watering Regimes

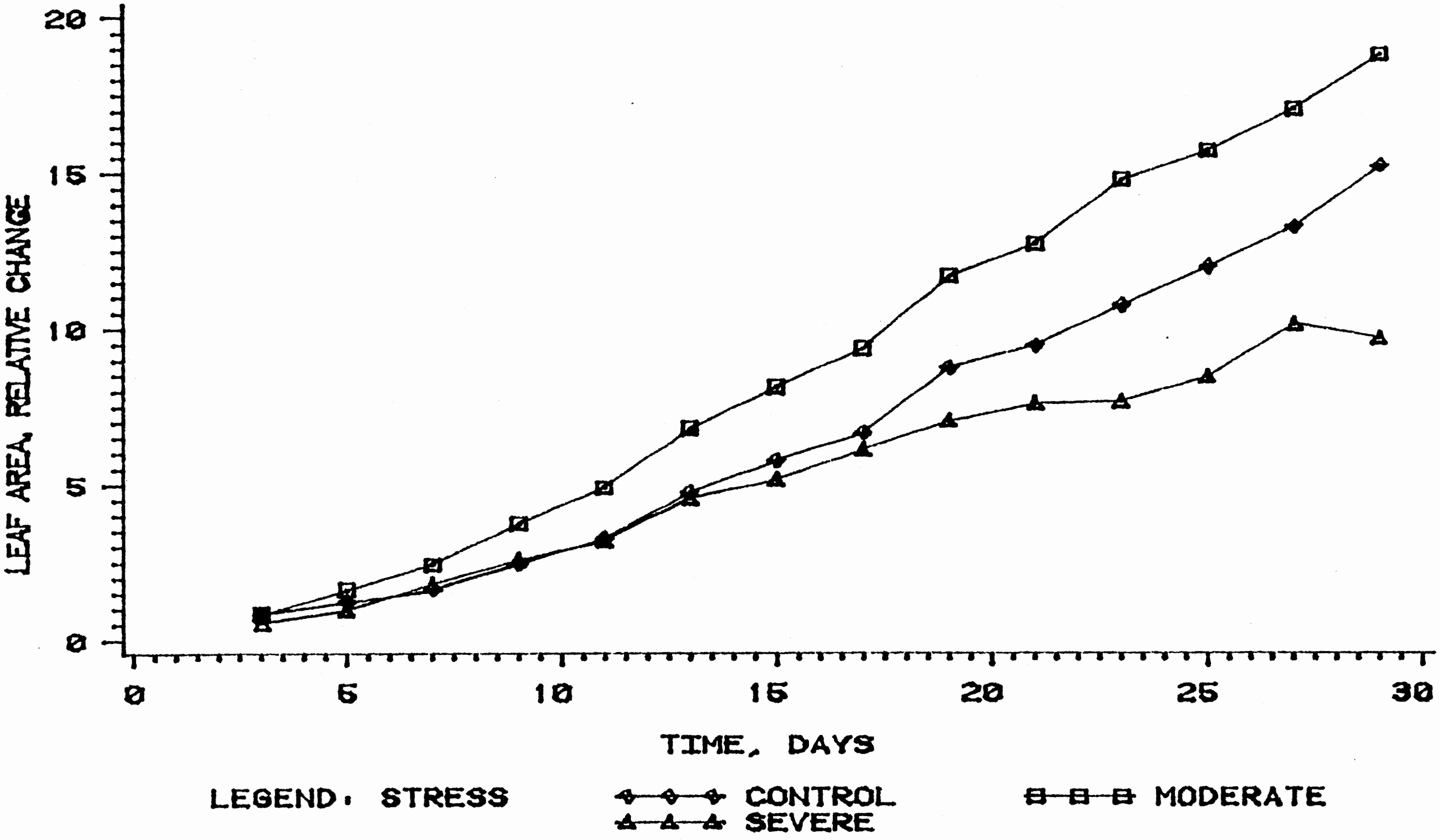

Figure 18. Relative Change in Leaf Area of Seaside Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

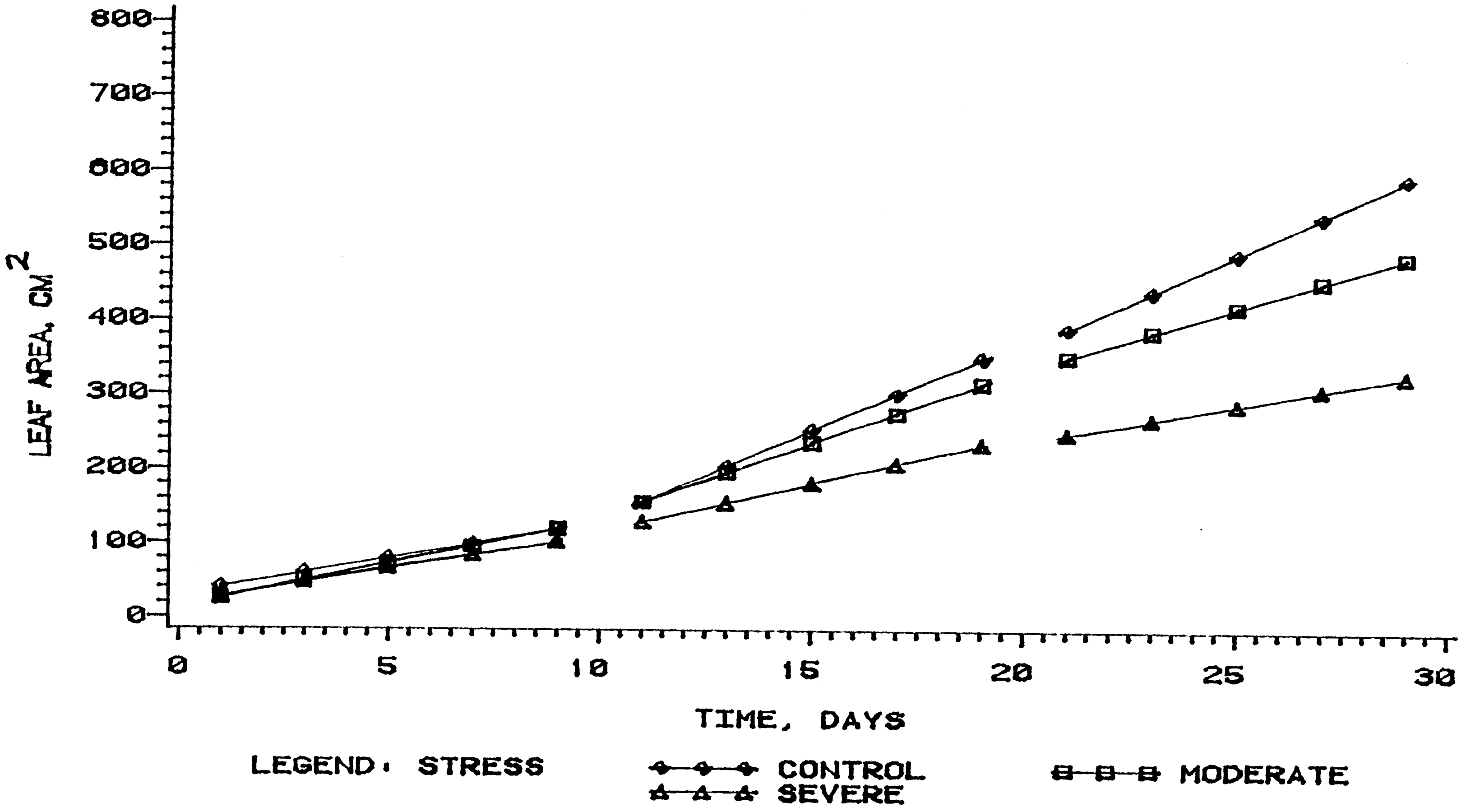

Figure 19. Plot of Regression Lines by Period for Leaf Area of Seaside Alder Under Three Watering Regimes

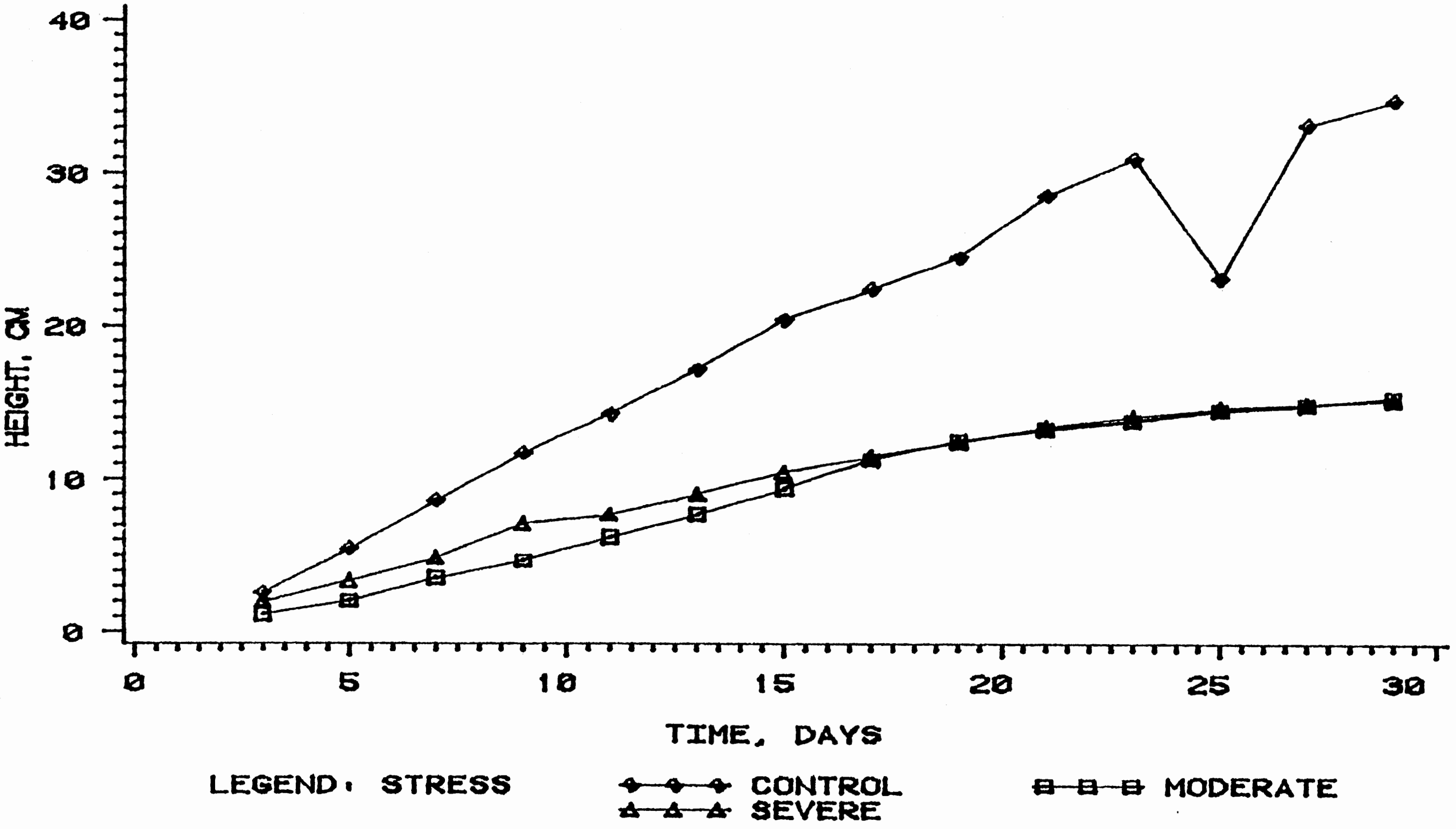

Figure 20. Absolute Change in Height of European Black Alder Under Three Watering Regimes

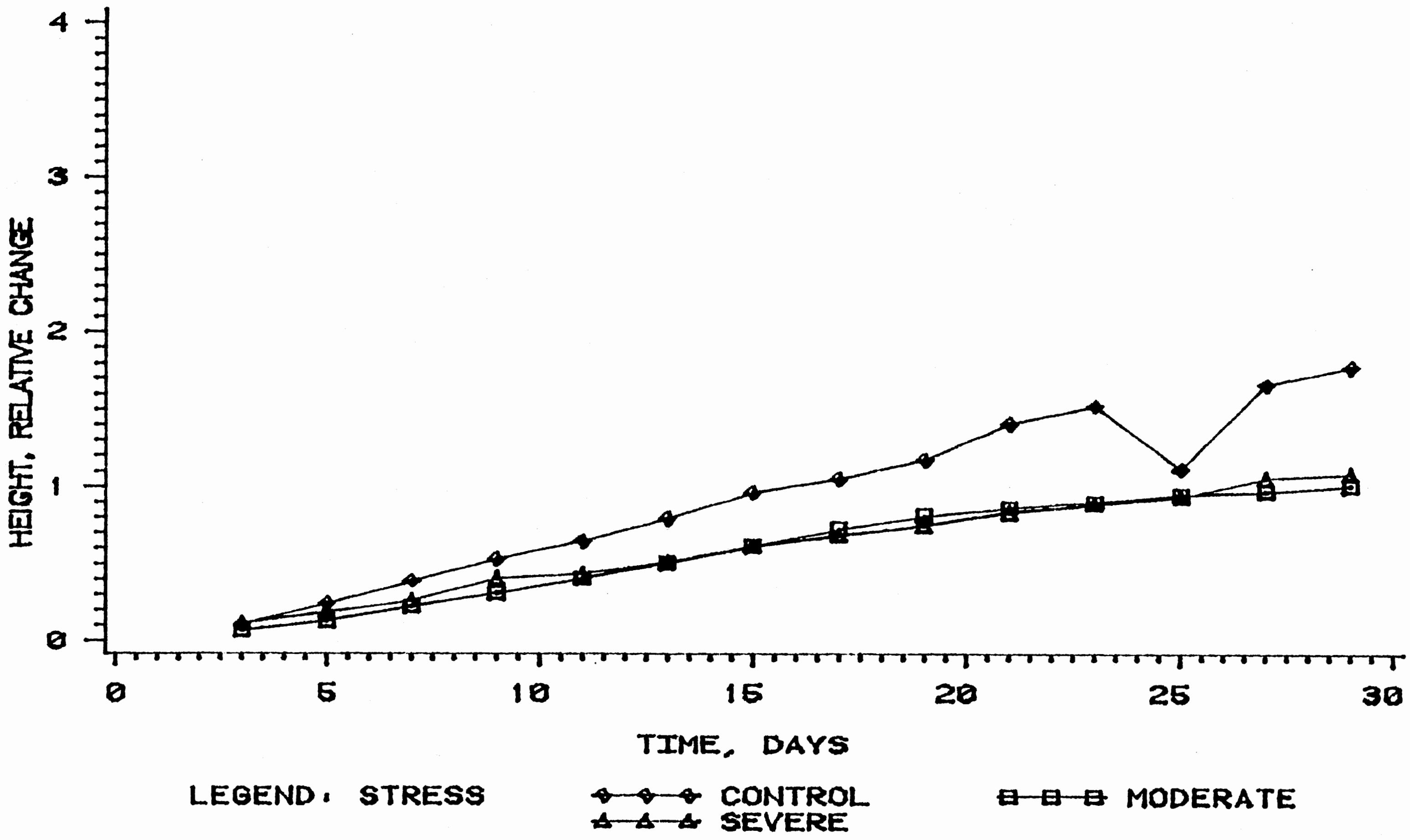

Figure 21. Relative Change in Height of European Black Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

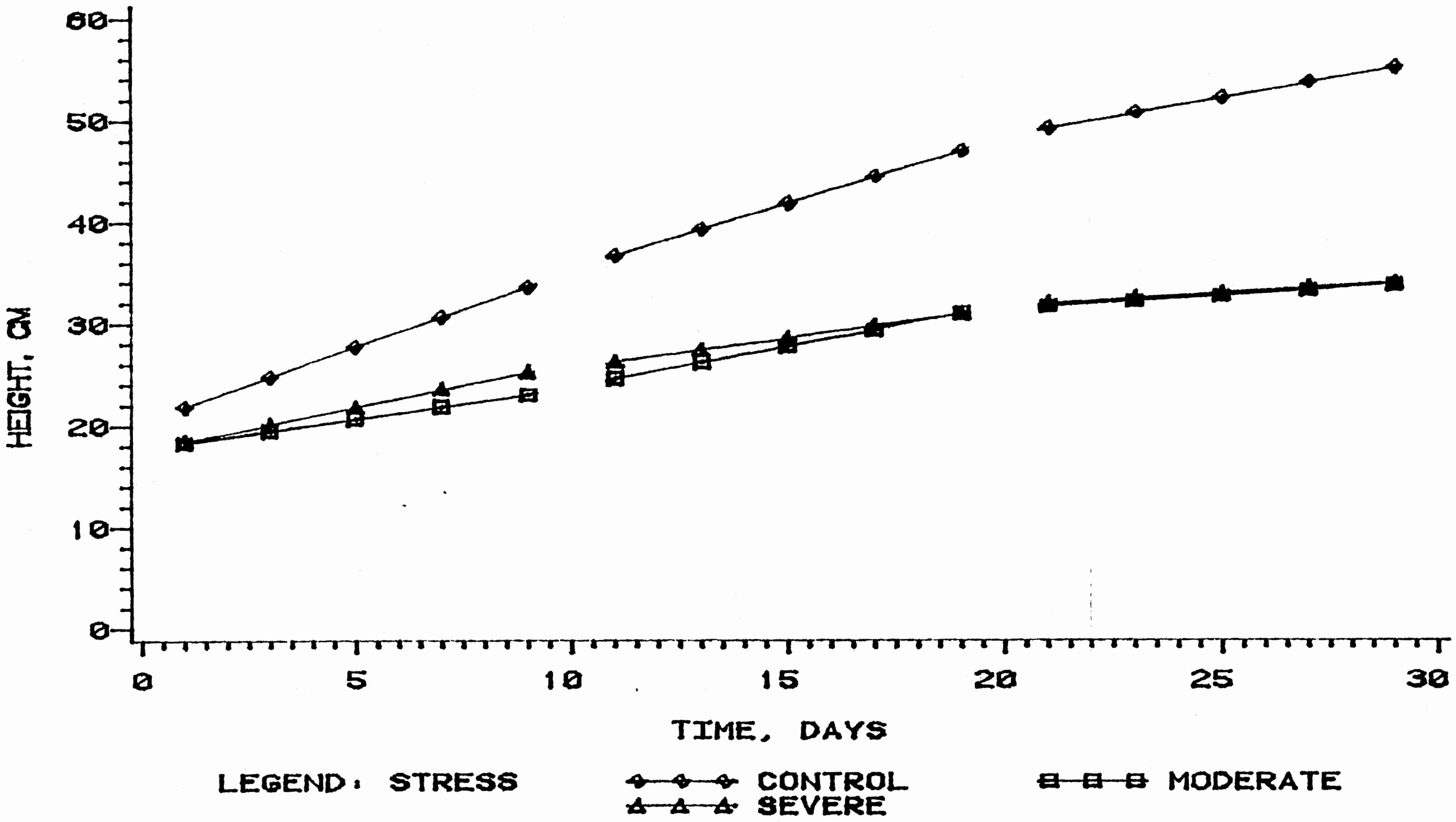

Figure 22. Plot of Regression Lines by Period for Height of European Black Alder Under Three Watering Regimes

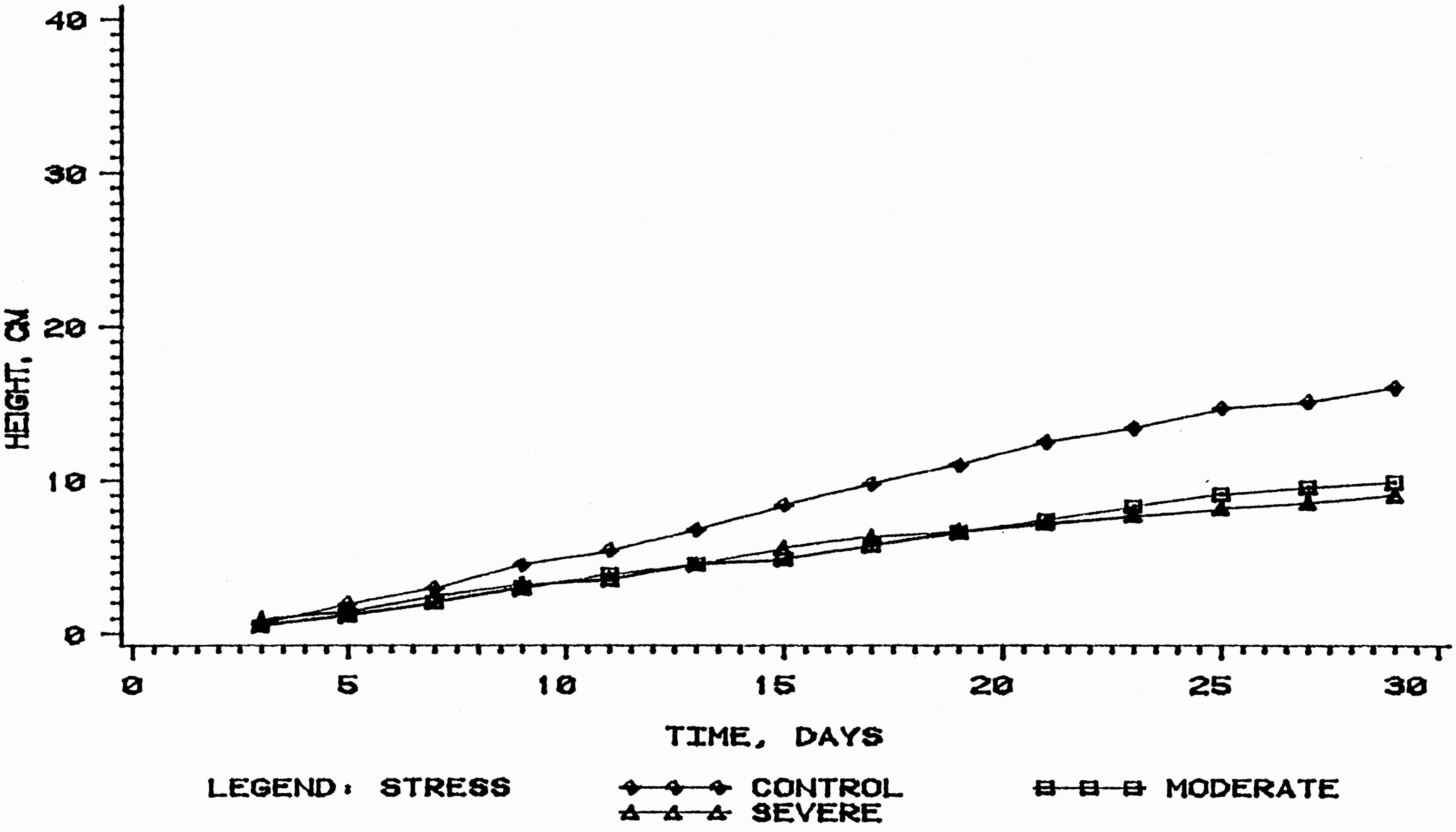

Figure 23. Absolute Change in Height of Hazel Alder Under Three Watering Regimes

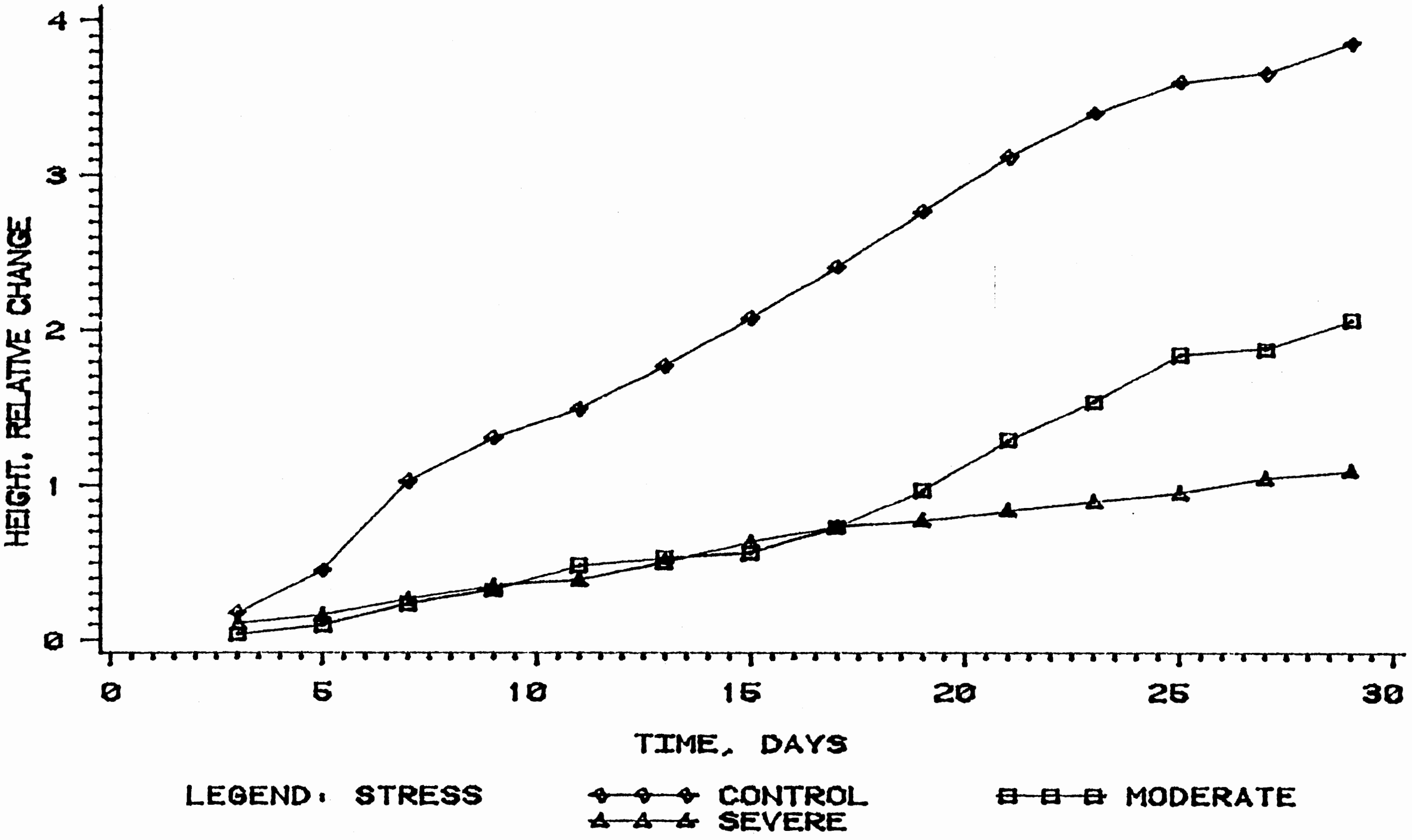

Figure 24. Relative Change in Height of Hazel Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

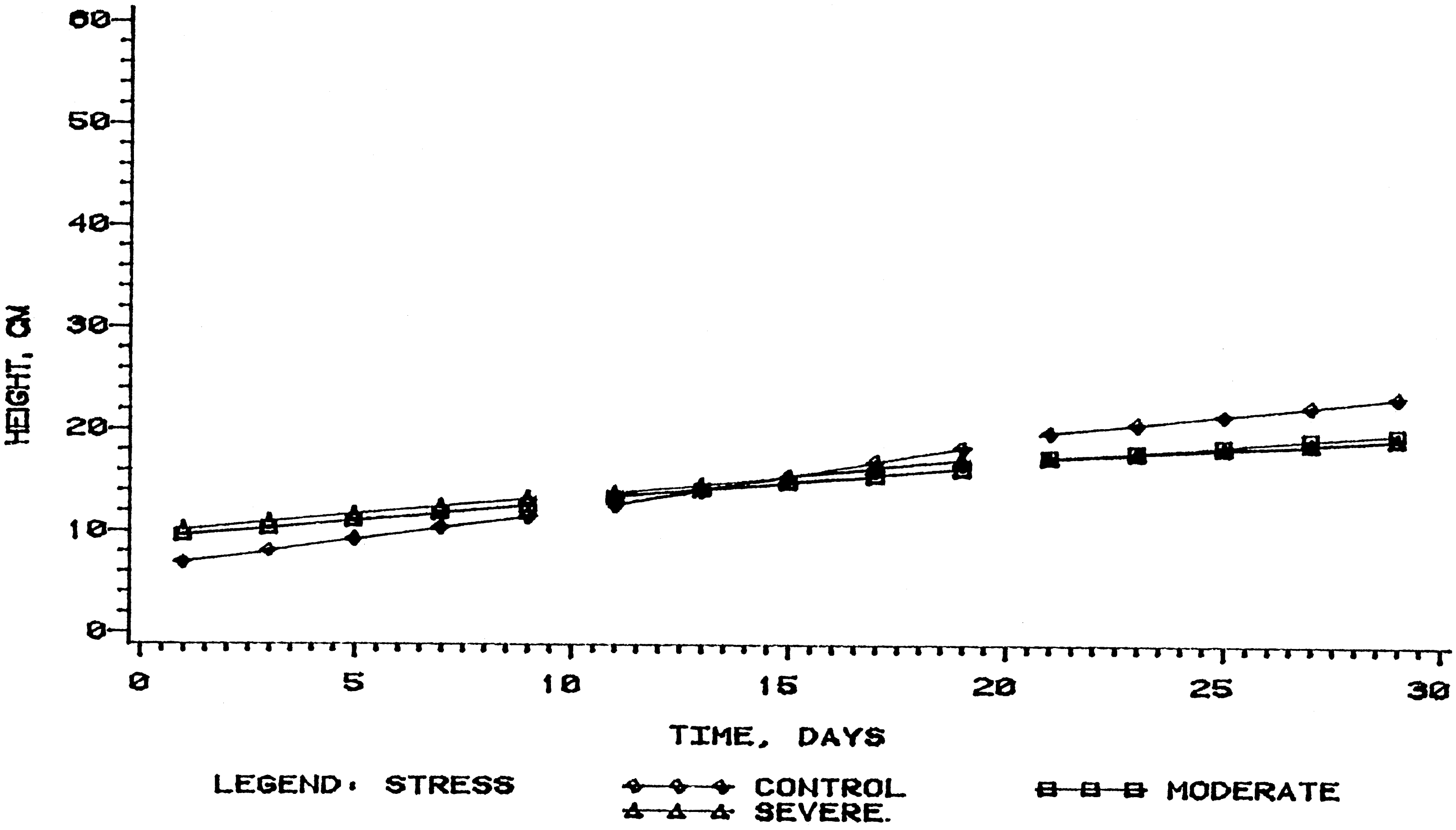

Figure 25. Plot of Regression Lines by Period for Height of Hazel Alder Under Three Watering Regimes

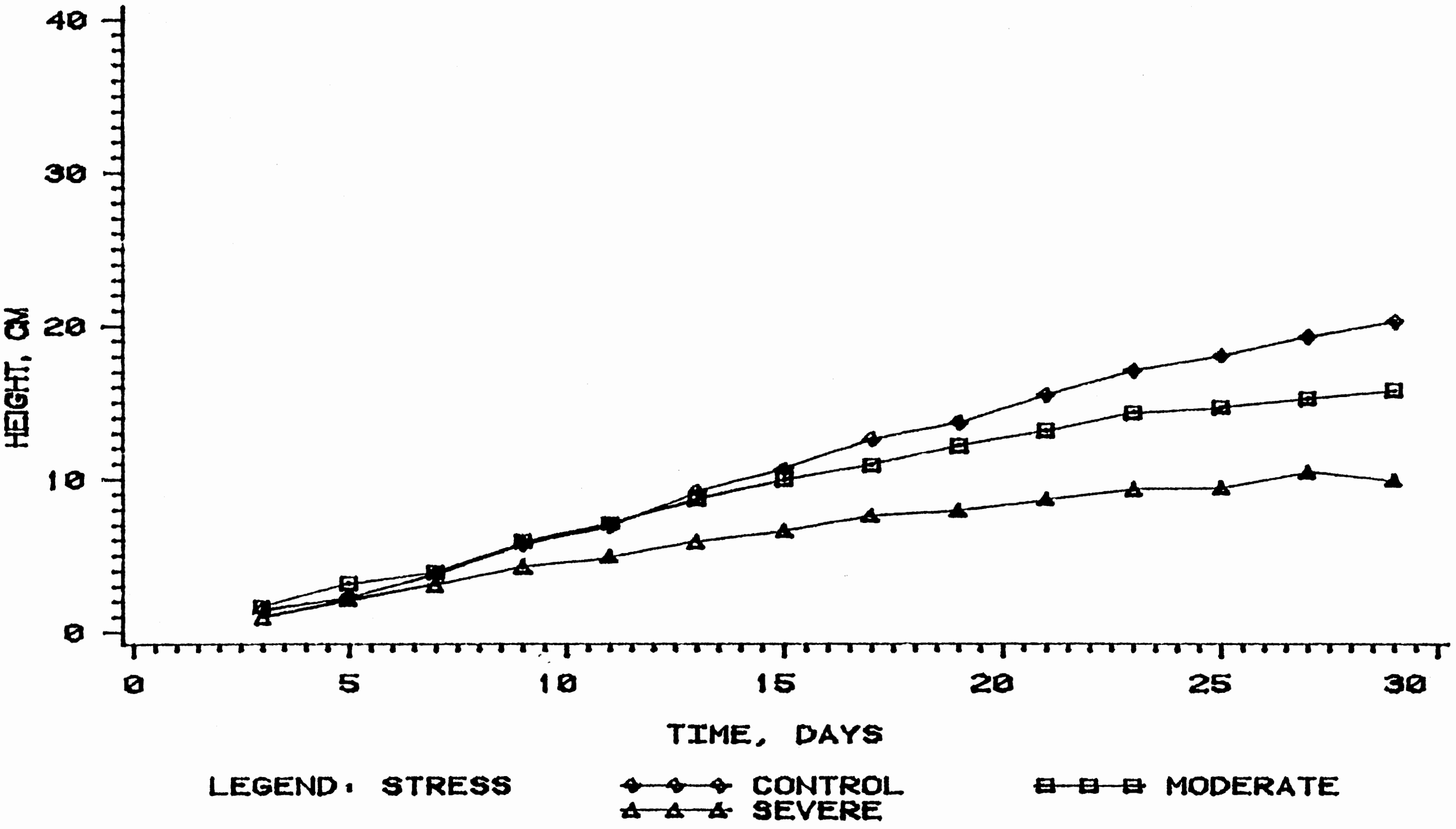

Figure 26. Absolute Change in Height of Seaside Alder Under Three Watering Regimes

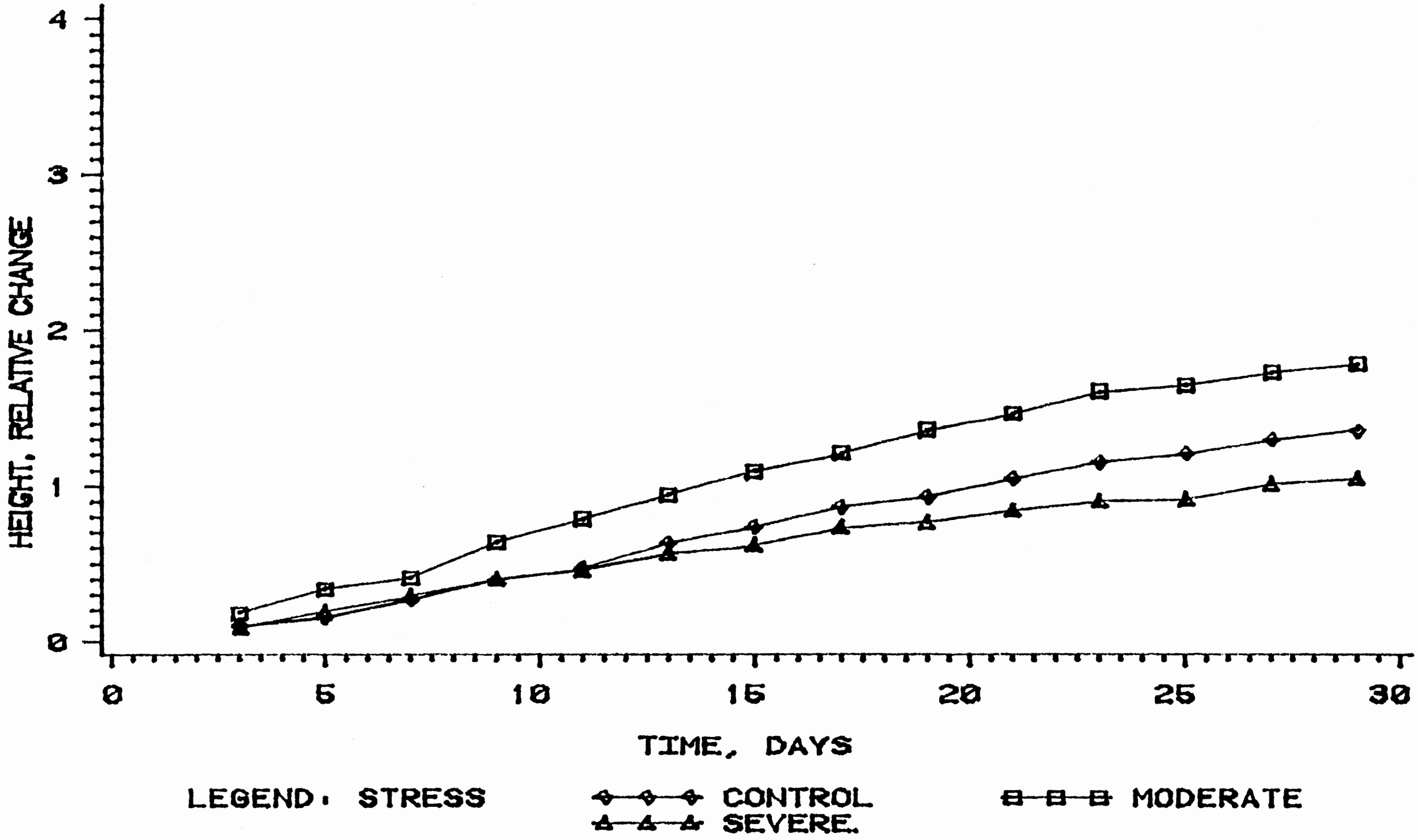

Figure 27. Relative Change in Height of Seaside Alder Under Three Watering Regimes. Percent Change Can Be Obtained by Multiplying the Values on the Vertical Axis by 100.

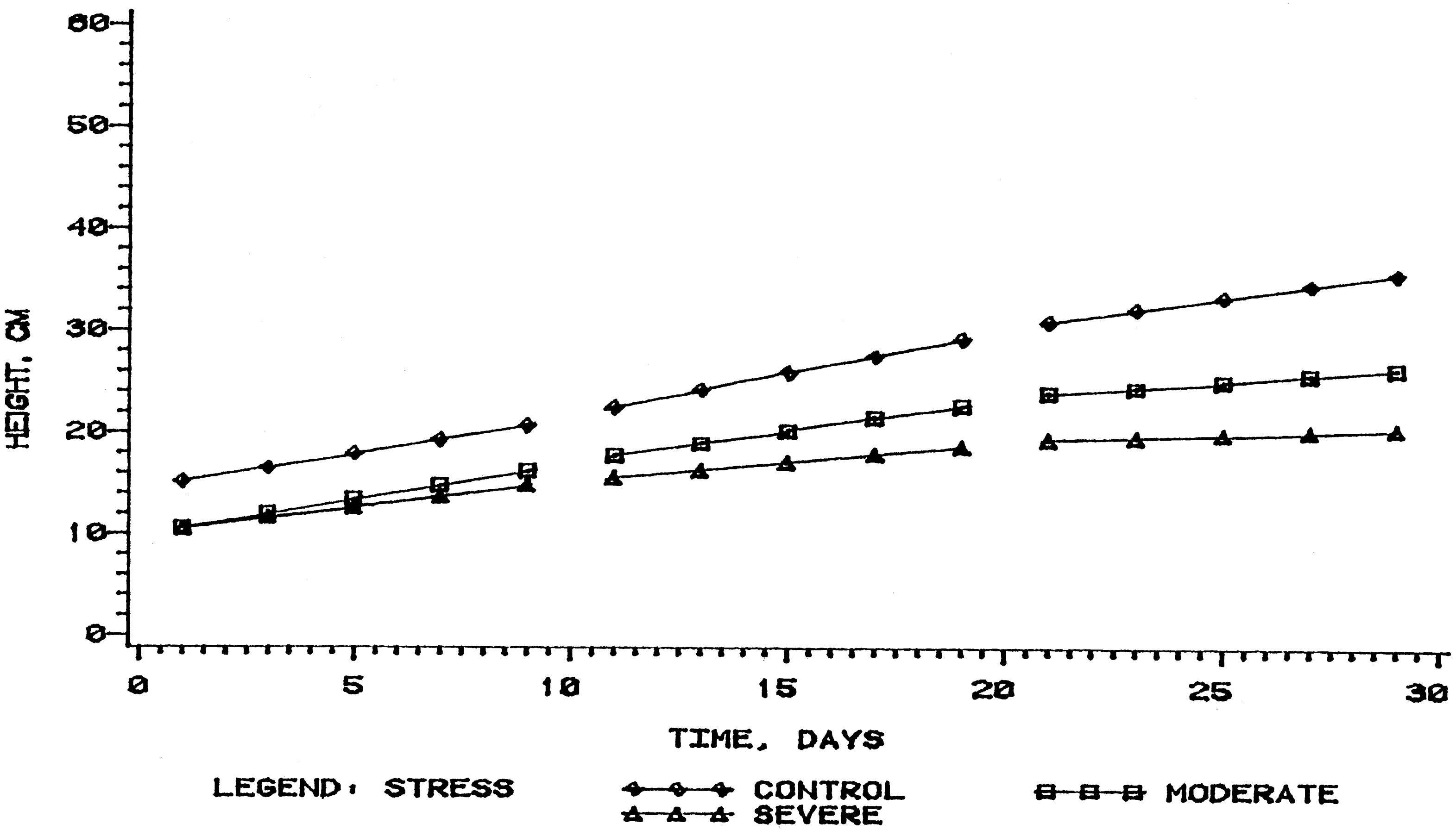

Figure 28. Plot of Regression Lines by Period for Height of Seaside Alder Under Three Watering Regimes

# VITA 2

Larry Keith Bair

Candidate for the Degree of

Master of Science

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