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Both red (*Trifolium pratense* L.) and white (*Trifolium repens* L.) clover seed yields can be highly variable and low in western Oregon. The objectives of this study were to: i) determine crop water requirements and supplemental irrigation timing, and ii) quantify the effects of soil and water status on inflorescence production, seed yield and seed yield components for red and white clover seed crops. In each species, five supplemental irrigation treatments were applied in 1990 and 1991 to first and second year seed crops grown on a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll) near Corvallis, OR. Non-irrigated controls were also maintained.

In red clover, increased plant water stress reduced the duration of the season-long bud and flower production, stem length, potential floral capacity (PFC), and seed yield (SY). Root rot index (RRI) increased with increasing levels of plant water stress, indicating that supplemental water applications reduced second-year root rot severity. The reduction in SY from increasing plant water stress was primarily caused by a decrease in floral fertility, and less conclusively by reductions in inflorescence number per unit area. One irrigation to fill the soil active profile during peak flowering provided adequate water to maintain efficient seed production.

In white clover, SY was maximum in 1990 when water application was delayed until 68% of the available soil-water was used by the crop which maintained an even flush of flowers and restricted vegetative growth. In 1991, all irrigation treatments yielded the same or less than the non-watered control. This was due to the excessive vegetative growth from stolons that had grown between the planted rows the previous and present crop year. In both years excessive amounts of irrigation water favored profuse vegetative growth and reduced SY. Inflorescence density was increased by constraining soil-water in 1990 and was the yield component that most affected SY both years.

Crop water stress index (CWSI) was a useful indicator of plant stress status and can be used to schedule irrigations in red and white clovers grown for seed under typical climatic conditions of western Oregon.

Water Relations in Red and White Clover Seed Crops

by

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

WATER RELATIONS IN RED AND WHITE CLOVER SEED CROPS

CHAPTER 1

GENERAL INTRODUCTION

Seed production of perennial clovers in the USA is concentrated in the irrigated areas of the arid western states. Red clover (*Trifolium pratense* L.) is an exception with more than 50% of the seed produced as a secondary crop to forage in the humid states of the Midwest. Annual production is about 12,500 t from 202,000 ha (USDA, Crop Reporting Board, 1980). In Oregon, red clover seed production is considered an agriculture specialty. During the last 10 years, seed production has exceeded 8,000 ha annually at an average yield of 410 kg ha^{-1} (OSU Extension Service, 1981-1990). White clover (*Trifolium repens* L.) seed is primarily produced in northern California, and much less importantly in Oregon, Washington and Idaho. A small amount of seed is harvested from pastures in the southeastern states. Ladino white clover seed produced in California represents almost all U.S. production. Approximately 2,500 ha are grown at an average yield of 404 kg ha^{-1} (Marble, 1990).

There is limited information on water management and crop stress response in red clover. The highest seed yields in the western U.S. were reported to be obtained by frequent irrigations which kept plants growing vigorously throughout the vegetative and seed setting periods (Fergus and Holowell, 1960; Rincker and Rampton, 1985). In New Zealand, irrigation after the removal of grazing pressure (closing) was reported to increase vegetative growth, reduce flowering, and was considered detrimental to seed yield (Clifford and Anderson, 1980). Most of the red clover seed crops are grown without irrigation water in western Oregon. This area receives large amounts of precipitation compared to other western U.S. seed production regions, but available soil water during the summer seed production period often becomes limited. This may contribute greatly to the inconsistencies in seed yield observed in the state.

In most seed-producing areas of California, Ladino clover requires from 900 to 1500 mm of irrigation water annually (Marble et al., 1970). No information is available regarding white clover seed water use in Oregon. In general, excessive foliage growth resulting from irrigation in summer can reduce both inflorescence density and floral fertility (Clifford, 1985). Constraining soil water during flowering reduces leaf size so more inflorescences are formed which produce higher seed yields (Zaleski ,1966; Clifford, 1986a; Daynach-Deschamps and Wery, 1987; Bullita et al.,1988).

Irrigation management can substantially increase seed yields in some forage legumes by increasing inflorescence density and floral fertility such as in white clover (Clifford, 1987) or increasing floral fertility as in alfalfa (Cohen et al., 1972; Steiner et al., 1992). The effect of water management on seed yield components for red clover has not been documented.

Soil-based methods have been the most-used criteria for irrigation timing (Stegman, 1983). However, since many soil-plant factors affect functional root system development, it is sometimes difficult to relate soil water content to crop yields. Consequently, scheduling irrigation on the basis of measured plant water stress appears to be a superior method since plant responses to both aerial and soil environments are measured (Jackson, 1981).

These studies were conducted to: i) determine crop water requirements using plant- and soil-based methods to control irrigation timing, and ii) quantify the effects of irrigation management on red and white clover inflorescence production and development, seed yield, and yield components in western Oregon.

The results are presented in the form of four chapters. Chapters 3 and 5 present calculations of the crop water requirements and compare the use of plant- and soil-based methods for scheduling irrigation in red and white clovers, respectively. Chapters 4 and 6 are concerned with the influence of plant water status on floral development, seed yield, and yield components of red and white clovers, respectively.

CHAPTER 2

LITERATURE REVIEW

Although red clover is a perennial species, it is considered a short-lived perennial because of the rapid decline in stand productivity. Red clover plants are erect to decumbent, with roots that penetrate deep into the soil. Plants form strong rosettes during the vegetative growth phase. Most varieties require exposure to long daylengths to initiate flowering (Griffiths et al., 1978). Plants generally do not require low temperatures during short days for successful floral induction. Flower buds are developed both terminally on the primary stems and on the branches from these stems, or on secondary branch nodes. Inflorescences bear large numbers of fully functional florets, each of which is capable of setting up to two seeds. Red clover is highly self-incompatible and sets seeds almost entirely by insect cross-pollination (Taylor and Smith, 1979)

White clover is a perennial species with rhizomatous, prostrate solid stolons which can root at the nodes (Gillett, 1985). Unlike red clover, white clover has a continuing process of cell division and expansion at the apical meristem. Axillary buds form in leaf axils. These may develop into either an inflorescence or a secondary stolon, but never both at the same site (Thomas, 1961, 1980). The change in axillary bud from secondary stolon to inflorescence formation is controlled by the inductive interaction of short day lengths and low temperature (Thomas, 1980, 1981a). Most white clover varieties have a daylength requirement of 15 h or more to initiate flowering. Inflorescences are borne on peduncles that grow up almost vertically from the stolon. The carpel normally contains 3-7 ovules, but only a few develop into seeds (Thomas, 1987). White clover flowers are morphologically similar to those of red clover, with the floral mechanism especially adapted to ensure cross-pollination by insects (Griffiths et al., 1978).

Effects of soil water availability on seed yield. In red clover, there is limited information for seed crop response to water management. Highest seed yields in the western U.S. were reported to be obtained by frequent irrigations which kept plants growing vigorously throughout the vegetative and seed setting periods (Fergus and Holowell, 1960; Rincker and Rampton, 1985). In New Zealand, however, irrigation after the removal of grazing pressure (closing) increased vegetative growth, reduced inflorescence density, and was considered detrimental to seed yield (Clifford and Anderson, 1980). The root and crown rot complex, caused primarily by several *Fusarium* species, is considered to be the most limiting factor for the continued productivity of forage stands and the main reason why seed crops cannot produce a satisfactory yield beyond the year after seeding (Leath, 1985).

In white clover, the reproductive expression depends on basic vegetative growth functions (Clifford, 1980, 1985, 1986a). All the stolons that have developed in leaf axils will contribute to forage yield. However, throughout the reproductive phase, a proportion of stolons is insufficiently developed to produce inflorescences. Nevertheless these will still grow and compete for space with reproductive stolons. In general, excessive foliage growth resulting from irrigation in summer can reduce both inflorescence density and floral fertility (Clifford, 1985). In white clover, reduced soil-water availability is considered the easiest way to control the decline in seed yield in high fertility soils (Clifford, 1987). Constraining soil water reduces leaf size so more inflorescences are formed which result in higher seed yields. Crop water requirements for maximum seed yield are less than that needed for maximum vegetative growth (Clifford, 1986b). The amount and timing of water application are important for regulating water stress conditions to provide the best compromise between inflorescence density, developing seeds, and vegetative growth (Clifford, 1987). Hagan et al. (1957) found that most inflorescences were formed in Ladino white clover when soil-water availability was maintained at 25% of field capacity. Zaleski (1966) found that irrigation was beneficial to seed production when applied between the period of removal of grazing livestock and the start of flowering, but irrigation during the flowering period reduced seed production. Adachi and Suzuki (1968) observed that high soil water tended to reduce inflorescence density and accelerate vegetative growth. Clifford (1986b) replaced available soil water to 50% of soil capacity each time 'near wilting' was reached from near peak flowering onwards. This system maintained mean plant available soil water at about 25%. Compared with non-irrigated treatments, irrigation increased yield by 53%. Daynach-Deschamps and Wery (1987) found that a 20% decrease in the amount of applied water during the reproductive phase induced more flowering, increased root and stolon biomass, and reduced both number and phytomass of leaves. Similar results were obtained by Bullita et al. (1988) who found that 20% reduction in applied water from the appearance of the first open bloom until the appearance of the first ripe seed head, substantially increased seed yield by increasing inflorescence density when compared to a range of treatments from non-stressed to non-irrigated.

Second-year white clover seed crops normally have too high a stolon density to optimize inflorescence production. In irrigated areas, appropriate water management throughout the season can help control the balance between vegetative and reproductive growth in white clover seed crops (Clifford, 1987). Other cultural techniques have been recommended to increase inflorescence production: i) mechanical gapping (removal of stolons) of the seed crop (Lay, 1980; Marshall, 1988), ii) defoliation by cutting, grazing and chemical defoliants (Clifford, 1979; Thomas, 1981c; Marshall et al., 1986, Marshall et al., 1989; Hollington et al., 1989), iii) regulation of plant density (Clifford, 1977, 1985; Marshall and James, 1988), iv) regulation of plant nutrition (Clifford and Rolston, 1989), and v) application of growth regulators (Marshall et al., 1986; Marshall and Hides, 1991a,b; Rijckaert, 1991).

Effects of soil water availability on seed yield components.

The final yield result of a seed crop is a direct consequence of two developmental stages: establishment of the yield potential, and yield potential utilization (Hampton, 1990). Forage legume seed yield potential is defined as the number of ovules (or potential seed sites) per unit ground area at anthesis (Lorenzetti, 1981). Although, extensive information is available to consistently produce crops with high yield potential, little is known about developmental and environmental requirements to maximize yield potential utilization (Thomas, 1987; Hampton, 1991).

The establishment of the yield potential is mainly dependent on the number of inflorescences per unit area and the number of florets per inflorescence (Hampton, 1990). In white clover, the potential number of inflorescences produced per unit area is determined by the number of growing stolon tips, including laterals, in that area. Inflorescence potential is fully utilized only when stolon numbers are insufficient to completely utilize the available space (Clifford, 1987). During first-year canopy formation, reduced soil water availability during the reproductive phase limits plant nutrient uptake and reduces leaf size (Clifford, 1979, 1986b). Consequently, inflorescence density is increased under a deficit irrigation regime. To the contrary, decreased soil water availability reduces number of florets per inflorescence because of nutritional deficiency at inflorescence formation (Clifford, 1979). This reduction was completely offset by the increase in inflorescence density which gave higher seed yields (Clifford, 1979, 1986b).

Utilization of the yield potential is determined by events at and after anthesis such as pollination, fertilization, and seed growth. These processes determine the number of seeds per floret and unit seed weight (Hampton, 1990). In white clover, the proportion of ovules that sets seeds is normally low (Thomas, 1987). The causes of such low seed set are not known. However, a combination of high temperatures and decreased soil water availability reduced the number of ovules per carpel from 5.9 to 4.5 after midsummer (Thomas, 1981b). Clifford (1986b) found that under deficit irrigation, ovule abortion was reduced 27% and seed weight increased 4% compared to the non-irrigated conditions.

Timing of irrigation and quantification of plant water stress. Quantification of plant water stress effects at field level is a difficult task. Stress responses are typically caused by more than one factor and their relative influences are difficult to isolate and quantify. Efforts oriented to determine an estimator of water stress and the best timing of irrigation are based on three different approaches: i) soil-based, ii) meteorology-based, and iii) plant-based (Jackson, 1981). Combinations of the three approaches are sometimes used. An example of a soil-based technique is the monitoring of soil-water content in the field. Knowing the field capacity (soil water content of the active profile 48 - 72 h after irrigation was ended) and the permanent wilting point (water remaining in the sample after being subjected to 1.5 MPa of air pressure) of the field soil, soil-water content information allows estimation of the amount of water lost in evapotranspiration and drainage below the active profile. When the soil-water content falls to a certain value, the amount of water required to bring the soil profile back to field capacity is added by irrigation. Although there are various measurement techniques for soil water content (e.g., gravimetric, tensiometric, and gypsum block), the neutron probe technique has proven to be the most practical and effective (Cuenca, 1988). Application of neutron probe readings with a calibration equation gives measures of soil-water integrated over a spherical soil volume approximately 40 cm in diameter.

A hydrological balance model is required that incorporates measured irrigation and precipitation amounts in order to estimate evapotranspiration of the crop. Although the neutron probe allows a practical monitoring of the full extent of the active soil profile, soil variability can cause significant errors in estimates of average field conditions (Jackson, 1981). Furthermore, since the only plant response parameter used in this approach (permanent wilting point estimation) is indirect, it is difficult to relate soil-water to crop responses such as yield. Thus, soil water conditions are not necessarily related to plant water status. Plant characteristics such as root depth, root density, genetically controlled water use syndromes (e.g., xerophyty) will lead to completely different plant responses under the same soil-water contents. Also, atmospheric conditions could impose temporary stress when the demand surmounts root-water uptake.

Many meteorological methods (Soil Conservation Service-modified Blaney-Cridle, FAO-modified Blaney-Criddle, pan evaporation, Penman, FAO-modified Penman, Wright Penman) have been developed (Cuenca, 1989). These methods use air temperature, net radiation, vapor pressure, and wind speed to model the amount of water evapotranspired during a given time period. The drawbacks to these methods are there is no direct account for drainage below the root zone and no direct plant information other than crop coefficients, which results in imprecise relationships between plant-water status and plant responses (Jackson, 1981).

Plant parameter direct measurements appear to be superior to indirect methods for quantifying water stress and timing irrigation since the plant responds to both its aerial and soil environment. Certain methods are limited to measurements on individual plant parts such as leaves and petioles. The pressure chamber (Scholander et al., 1965) measures the xylem pressure (Pxylem) averaged over freshly severed leaf with petiole placed in the chamber with the cut end protruding through a rubber seal. Air pressure (Pair) in the chamber is then gradually increased until it causes the exudation of xylem sap at the cut end. The resulting pressure of the sap, which equals Pxylem + Pair, is zero, and so Pxylem = - Pair. If xylem osmotic pressure is considered negligible, Pxylem approximates plant water potential (Ψ_p). The leaf diffusion porometer (Kanemasu, 1975) is a small chamber (cuvette), often only 1 to 2 cm in diameter, which is clamped for a short time on a leaf surface (usually the lower surface, where most stomates are located), and humidity inside the chamber is monitored to measure transpiration. Most recently, steady-state porometers have become commercially available (Salisbury and Ross, 1992). Air is passed through a drying column and introduced into the chamber at a rate exactly sufficient to maintain humidity in the chamber at its initial value. A microprocessor calculates transpiration from the absolute humidity (relative humidity and air temperature) and the rate at which dry air must be introduced to maintain constant humidity. These methods are time consuming and require numerous repeated measurements to characterize an entire field. Moreover, the variability of point measurement of plant properties such as plant water potential is considerable (Jackson, 1981).

Modern infra-red thermometry obviates this disadvantage by rapidly surveying a large number of plants and integrating plant temperatures over entire fields or characteristic sections of fields (Jackson, 1981). This technique is based on the relationship between canopy temperature as measured by an infrared thermometer and air temperature. If the differential between canopy and air temperatures is negative, the plants are well-watered, but if the differential is positive, the plants need water. Crop water stress index values are estimated when measured differentials are scaled relative to the differential expected under potential evapotranspiration (non-water-stressed conditions) and the maximum differential occurring under completely suppressed evapotranspiration (fully stressed conditions). The scaled values are normalized for environmental variability through their relationship with the air vapor pressure deficit (Idso et al., 1981).

CHAPTER 3

CROP WATER REQUIREMENTS AND IRRIGATION TIMING FOR RED CLOVER SEED PRODUCTION

ABSTRACT

Red clover (*Trifolium pratense* L.) seed production water management and crop stress response information is limited and not well defined. This study was conducted in 1990 and 1991 to determine crop water requirements, and evaluate crop water stress index (CWSI) and fraction of available soil water used (FAWU) for timing supplemental irrigation. Six irrigation treatments were used to assess the influence of within- and between-season crop water requirement changes on calculated CWSI and FAWU at Corvallis, OR, on a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll). In the non-stressed treatment, the soil water content was brought to field capacity by twice weekly replacement of water used since the last application. Two treatments had post-haying water replacement to 100% of field capacity, one of which again had the soil profile replenished to 100% field capacity at peak flowering time. Two treatments received single applications of water which brought the soil water content to 50 and 100% of field capacity at peak flowering. A non-irrigated control was also maintained. Two distinct non-stressed baselines for canopy-air temperature differences versus vapor pressure deficit were identified that were related to increasing leaf senescence and internal hydraulic resistance during crop ageing. CWSI values across all treatments were negatively related (r^2 = 0.75) to plant water potentials. Root deterioration caused by root and crown rot complex was observed to have affected all red clover plants after the 1991 seed crop was harvested. As a consequence, FAWU values were generally much lower in 1991 than in 1990. CWSI values at the times of irrigation were consistently similar within each treatment for both years, indicating that root rot damage rather than available soil water was limiting plant water uptake in the second year crop. Consequently, water use efficiency was

generally reduced in 1991. During each season, CWSI generally increased faster than FAWU. Unlike FAWU, CWSI integrated the plant total environment, detecting within-season changes in vascular resistance as well as reduced water uptake due to damaged root tissues by root rot the second year. CWSI is a useful indicator of plant stress status and can be used to schedule irrigations in red clover grown for seed under typical climatic conditions of western Oregon.

INTRODUCTION

Red clover, alone or in grass mixtures, is a widely grown forage legume in the USA and Canada. Red clover seed production is a specialized industry in western Oregon. This area receives large amounts of precipitation compared to other western U.S. seed production regions, but available soil-water during the summer seed production period often becomes limited.

There is scant information about red clover seed production water management and crop water stress response. Highest seed yields in the western U.S. were reportedly obtained by frequent irrigations to keep plants vigorously growing throughout the growing and seed setting periods (Fergus and Holowell, 1960; Rincker and Rampton, 1985). In New Zealand, however, irrigation after the removal of grazing pressure (closing) increased vegetative growth, reduced flowering, and was considered detrimental to seed yield (Clifford and Anderson, 1980).

Soil-based methods have been the most-used criteria for irrigation timing (Stegman, 1983). However, since many soil-plant factors affect the plant ability to develop a functional root system, it is sometimes difficult to relate soil water content to crop yields. Consequently, scheduling irrigation on the basis of measured plant water stress appears to be a superior method since plant responses to both aerial and soil environments are measured (Jackson, 1981). This experiment was conducted to determine crop water requirements for red clover grown for seed using plant- and soil-based methods to control irrigation timing.

Abbreviations: ASWU, average seasonal water use; CWSI, crop water stress index; DOY, day of year; ET_c, estimated crop evapotranspiration; ET_r, grass reference evapotranspiration; FAWU, fraction of available soil water used; Ψ_p, plant water potential; T_a, air temperature; T_c, canopy temperature; VPD, air vapor pressure deficit.

MATERIALS AND METHODS

The experiment was conducted as a randomized complete block design with four replications and six treatments for two years at the Hyslop Research Farm near Corvallis, OR. The soil was a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll).

Each experimental unit (EU) was 15 rows 10 m long (45 m^2) and isolated by a furrow-dike system to prevent lateral surface water movement when application rates exceeded infiltration rate. The experimental area was fumigated with methyl bromide (360 kg ha^{-1}) prior to seed bed preparation to uniformly control weeds. Medium red clover 'Kenland' (Hollowell, 1951) was sown 14 September 1989 in a level seedbed in single rows, 0.3 m apart, at a rate of 1.7 kg ha^{-1}. One 25-mm sprinkler irrigation was applied after seeding to establish the crop. The EUs were harvested for seed during August and September in 1990 and 1991.

Crop culture followed common commercial practices. All EUs were harvested for hay in the spring (DOY 162 in 1990 and 160 in 1991). Haying at early-flowering is an important operation. Following regrowth, blooming is better synchronized with warm weather when insect pollinators are fully active. Hay harvest also helps control some weed species and several insect pests. Aphids (*Nearctaphis bakeri* (Cowen)) and lygus bugs (*Lygus* spp.) were controlled with 0,0, dimethyl S-[2-ethylsulfinyl)-ethyl] phosphorothioate applied at bud stage of development at a rate of 1 kg ha^{-1}. Annual grasses and broadleaf weeds as well as volunteer clover seedlings were controlled in winter 1991 with diuron at a rate of 3.2 kg ha^{-1}. Four honey bee (*Apis mellifera* L.) hives were placed adjacent to the experimental area at the beginning of bloom time. Honey bee and naturally occurring bumble bee (*Bombus* spp.) activity was sufficient for adequate pollination.

All EUs had similar soil-water contents at the beginning of each cropping period because rainfall during the winter maintained soil water at or above field capacity. A surface trickle irrigation system consisting of a mesh filter, ball valve, residential water flow meter, volumetric controller, and pressure regulator, distributed water to each EU. Within each EU, water was delivered through five plastic trickle lines 0.9 m apart and placed parallel to the planted rows. The trickle lines were fitted with in-line turbulent-flow emitters spaced 0.9 m apart that delivered 4 L of water h^{-1}. Water was distributed to all four replicates of each treatment at the same time.

Five supplemental water application treatments were applied during the cropping period from haying to seed harvest. The treatments were: NS) non-stressed, the soil was brought to field capacity by twice-weekly replacement of the soil water used since the last application until 3 weeks before seed harvest; HH) two irrigations to refill the soil profile to 100% of field capacity, one when the soil water depletion was 20% after haying and the second at peak flowering; H0) single water replacement to 100% of field capacity when the soil water depletion was 20% after haying; 0H) single water replacement to 100% of field capacity at peak flowering; and 0L) single water replacement to 50% of field capacity at peak flowering. A non-irrigated control treatment (C) was also maintained. Peak flowering is defined as the time when the crop displays a maximum number of inflorescences in anthesis as determined by weekly observations.

Changes in volumetric soil water content were monitored weekly by neutron attenuation (Cuenca, 1988) using 0.08-m diameter aluminum access tubes placed in the center of every EU. The readings were made at seven depths from 0.45 to 2.45 m in the high water application treatments (NS and HH), and at six depths from 0.45 to 2.00 m in the remaining treatments. The neutron attenuation probe was calibrated to the local soil conditions using gravimetric samples taken at the time of access tube installation and throughout the duration of the experiment representing a range of readings (Fig. 3.1). An average soil bulk density value (1.35 g soil cm^{-3} soil $\pm$ 0.02) was obtained from five soil depths and used to convert gravimetric soil water content into volumetric values. The neutron probe counts were related to percent volumetric soil water content using the equation:

$$VWC = A\ (NMC/STD) + B$$

where VWC = volumetric soil water content (%), NMC = neutron meter count, STD = standard count for the particular neutron probe and, A, B = statistical calibration coefficients (Cuenca, 1988). Field capacity (46.03 cm^3 water cm^{-3} soil) was determined 48 h after an irrigation which refilled a 2.5-m profile. Permanent wilting point (21.5 cm^3 water cm^{-3} soil) was determined in the laboratory as the water remaining in a soil sample after being subjected to 1.5 MPa of air pressure.

Seasonal ET_c was determined by summing applied water, precipitation, and the change in soil water content measured by neutron probe. No deep percolation was assumed. Average daily ET_c was calculated by dividing seasonal ET_c by the crop season length in days. Seasonal reference evapotranspiration (ET_r) was determined from a class A evaporation pan located 200 m from the experiment site using a coefficient for different groundcover and levels of mean relative humidity and 24-h wind (Doorenbos and Pruit, 1977). Average seasonal water use (ASWU) was calculated as the ratio between seasonal ET_c and seasonal ET_r. FAWU was calculated as:

$$FAWU_i = 1 - (AW_i \ / \ TAW)$$

where $FAWU_i$ = fraction of available soil water used of sample i, AW_i = available soil water of sample i in mm, and TAW = total available soil water in mm; also:

$$AW_i = (VWC_i - PWP)\ D$$

where VWC_i = volumetric soil water content of sample i in cm^3 water cm^{-3} soil measured with a neutron probe, PWP = permanent wilting point, and D = depth of active soil profile in mm; and:

$$TAW = (FC - PWP)\ D$$

A Scheduler® Plant Stress Monitor (Carborundum Company, Solon, Ohio) measured crop canopy (T_c) and air (T_a) temperatures and air vapor pressure deficit (VPD). Four measurements per EU in all treatments were taken weekly (DOY 196 to 225 in 1990, and 184 to 234 in 1991) on sunny days between 1200 to 1400 h. Oblique measurements were taken about 1 m from the top of the canopy at a 45° angle facing north, from both sides of the longer east-west axis of the EUs. CWSI was estimated using the technique of Idso et al. (1981) which is based on the relationship between T_c and T_a. The measured differentials were scaled relative to the differential expected under potential evapotranspiration (non-water-stressed conditions) and the maximum differential occurring under completely suppressed evapotranspiration (fully stressed conditions). The scaled values were normalized for environmental variability with VPD. Data collected in NS treatment were used to determine the non-stressed baselines for the T_c-T_a versus VPD relationship.

The influence of inflorescences on canopy temperatures and calculated CWSI values was evaluated in 1991. Measurements were made on DOY 200, 207, and 214, on 1.0 m^2 sections of control EUs with 0, 25, 50, 75 and 100% of the inflorescences sequentially removed. Measurements were taken using the same methodology described above and the target was kept inside the sequential flower removed sample area.

Plant water potential (Ψ_p) measurements were made both years with a pressure chamber (Scholander et al., 1965). Three random leaves (third fully developed leaf from the top of a main stem) were sampled weekly from every plot between 1200 to 1500 h. In the second year, sampled leaves were wrapped in plastic clingfilm prior to excision and during measurement to minimize water loss error at excision (Leach et al., 1982).

Green leaf coverage was monitored weekly from peak flowering until harvest. Thirty samples per EU were taken with a cross-wire sighting device (Ghersa and Martinez-Ghersa, 1991) to monitor the presence or absence of green leaves in each treatment.

An 8-m section of the three center rows (7.2 m^2) of each EU was harvested with a gas-powered mower in early-morning when 80% of the florets were dry and able to shatter.The plant material was gathered by hand, put in burlap bags, and dried at 32 °C for 3 d. Above-ground phytomass was weighed and the seeds threshed, cleaned, and weighed. Total above-ground phytomass and seed yield water use efficiencies were calculated as the ratio of the component to seasonal ET_c.

All variables were tested by analysis of variance and simple linear regression analysis was used to relate VWC and NMC/STD ratio, T_a-T_c differential and VPD, and Ψ_p and CWSI. Standard error of the mean was calculated for VMC, FAWU and CWSI measurements. FAWU/CWSI ratio was converted to standard variates by subtracting the mean of each variate and then dividing by the standard deviation (Snedecor and Cochran, 1980). Student's t pairwise comparisons were used to contrast water use efficiency means between years 1990 and 1991. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Crop water requirements. The top 1.55 m from the soil surface were considered to be the active profile based on the soil water content changes (Fig. 3.2). All water balance calculations assumed that no deep percolation occurred from the time of haying until seed harvest. The soil water content at initiation of the irrigation treatments was higher in 1991 than in 1990 as a consequence of different levels of precipitation during March and April (336 mm and 153 mm, respectively). In 1990, 15% of the available soil water had been depleted at haying, while at that time in 1991 the soil was still at field capacity. Initial irrigation treatments were delayed in 1991 compared to 1990 until later in the reproductive period (Fig. 3.3).

Stored soil water depletion contributed more to the seasonal ET_c in 1991 than in 1990 due to higher soil water contents at haying. Total applied water was higher for all treatments in 1990 than in 1991 (Table 3.1).

Red clover root and crown rot complex was found to reduce vegetative production and seed yield in 1991 (Chapter 4). Root rot disrupted the vascular function of root tissues, constricting plant water uptake and reducing evapotranspiration. Seasonal ET_c and ASWU values for NS, HH and H0 treatments were lower in 1991 than in 1990, the opposite was observed for 0H, 0L and C treatments (Table 3.1). A general crop coefficient mean value of 1.05 has been cited for clover pasture crops grown in dry climates with light to moderate wind (Doorenbos and Pruit, 1977). Except for NS both years and HH in 1990, all other treatments had lower ASWU values than 1.05. Since seasonal ET_c varies with the length of the cropping season, daily ET_c is a better comparative estimator of the water used by the different treatments. Daily ET_c values were generally lower in 1991 than in 1990 (Table 3.1). More soil water was available in 1991 throughout the seed production period than in 1990 (Fig. 3.2). ASWU values were lower for NS, HH and H0 in 1991, indicating that the reduction in evapotranspiration was caused by root rot damage rather than soil- and atmospheric-induced stresses. Under late- and non-irrigation conditions (0H,0L, C) evapotranspiration was similar and minimized both years. Seed production, however, was reduced the second year in all treatments (Chapter 4).

As a consequence of root rot damage, water use efficiency was generally reduced in 1991 (Table 3.2). Total above-ground phytomass water use efficiencies decreased in 1991 compared to 1990, except for the NS and H0 treatments which had severe lodging in 1990. Seed yield water use efficiency was lower for all treatments in 1991. In both years, maximum water use efficiency for both total above-ground phytomass and seed yield was obtained with one irrigation scheduled at peak flowering time (0H and 0L). Increased replenished water amount increased seed yield 21% (0H > 0L; Chapter 4).

Plant- and soil-based methods as irrigation timing techniques. Two non-stressed baselines were used to determine CWSI values (Fig. 3.3). The functions of the fitted linear regression lines for the two stages of plant development were the same for both years. The in-season change in base line coincided with 50% maturity of inflorescences. Negligible changes in available soil water in the active profile during this period did not account for the increase with time in the T_c - T_a differential (Fig. 3.4). Measurements of canopy temperature for different densities of inflorescences were used to determine whether the presence of reproductive structures affected the T_c - T_a differential as suggested by results in alfalfa seed production (Hutmacher et al., 1991). Canopy temperature measurements taken before and immediately after the removal of immature and mature flowerheads were not affected by inflorescence density (data not shown). This suggests that changes in internal hydraulic resistance during crop ageing could be modifying the baseline. To make CWSI measurements comparative to one another during the entire season, all values were scaled to the highest theoretical upper line which resulted from the baseline with the function $T_c - T_a = 5.9 - 2.6$ (VPD) (Fig. 3.4).

Although the pressure chamber method is cumbersome and measurement variability great, it has generally been accepted as a fundamental measure of plant water status (Jackson, 1981; Turner, 1981). In agreement with the results cited in alfalfa seed studies (Hutmacher et al., 1991), Ψ_p values were linearly related with CWSI measurements, reflecting the usefulness of this index for quantifying plant water stress (Fig. 3.5).

FAWU generally increased with time with temporary decreases following irrigation (Fig. 3.3). Irrigation amounts, except in treatment 0L, were calculated to fill the entire active profile (FAWU = 0). This was not apparent with each water application since soil water status was determined only once a week. CWSI also increased with time but the values after an irrigation did not return to their same relative position with the FAWU values prior to the irrigation. Likewise, CWSI values in treatment C tended to increase faster than FAWU values as the season progressed. The general trend of CWSI increasing faster than FAWU was more discernible by the FAWU/CWSI ratio (Fig. 3.3). An exception to this trend was the CWSI decrease at the end of the crop cycle in 1990 (HH, H0, and 0H treatments). This could have been related to late-season low temperatures and precipitation that affected only the upper portion of the soil profile and which was undetected by the neutron probe.

Green canopy coverage successively decreased in all treatments both years from complete coverage at peak flowering time to about 50% coverage at harvest (data not shown). As green leaves began to senesce, the transpiration rate may have decreased together with evaporative cooling, as indicated by the increase in the non-stressed T_c - T_a differential. As a consequence, after an irrigation the canopy temperature remained higher than expected from the low FAWU. The effect of plant senescence on the FAWU-CWSI relationship agrees with findings for wheat (Jackson, 1981) and indicates ageing as the causal factor for baselines change.

CWSI values at the times of irrigation were consistently similar within each treatment for both years (Table 3.3). FAWU values, however, were generally much lower in 1991 than in 1990, indicating that factors other than available soil water were stressing the second year crop. Plants at the time of post-haying irrigation (HH and H0) were under mild water stress (1991, CWSI $\approx$ 0.09). HH and H0 reached peak flowering time with CWSI $\approx$ 0.18. Late-irrigated treatments (0H and 0L) exhibited a CWSI $\approx$ 0.28 at peak flowering time. One irrigation to 100% of field capacity at peak flowering (0H) was adequate to moderate water stress and lessen damage from root rot. This increased seed yield and water use efficiency both seasons for 0H. When the crop was subjected to even greater water stress levels (C), seed yield was reduced both years by more than one half in relation to treatment 0H (Chapter 4).

Unlike FAWU, CWSI values were practically the same among treatments that were expected to have similar water stress conditions (HH, H0, and 0H, 0L, C; Table 3.3). CWSI was a more realistic indicator of plant stress status than FAWU. Based on 1990 soil water availability results, scheduled water application at peak flowering time could have been delayed or avoided in 1991. However, CWSI revealed that the second-year crop root damage caused increased stress due to limited soil water uptake. This condition was not detectable with a soil based irrigation-scheduling technique.

CONCLUSIONS

CWSI is a useful indicator of plant stress and can be used to schedule irrigations in red clover grown for seed under typical climatic conditions of western Oregon. The use of soil water availability as a scheduling technique was not able to detect within-season changes in plant water requirement as well as plant stress from root rot that reduced water consumption the second crop year. FAWU values showed considerable variation among treatments which were expected to have similar soil water conditions based on the amount of water applied. Soil variability could cause significant errors in estimates of average field conditions. CWSI values expressed plant conditions such as varying vascular resistance during the season or reduced water uptake due to damaged root tissues by root rot. Therefore, CWSI can be used to optimize irrigation scheduling even in the presence of such factors. Moreover, CWSI values were consistent among similar treatments because of the rapid integration of canopy temperatures over the entire field plot rather than the limited sampling area used by neutron attenuation. CWSI measures the combined effects of soil conditions, atmospheric demands, and intrinsic plant conditions such as disease and phenologic development. Thus, a critical value of CWSI as an indicator of irrigation timing may vary as a consequence of the multiple factor effects and interactions to which the crop is subjected. Under the conditions of this experiment, a single irrigation (treatment 0H) filling the active soil profile at CWSI $\approx$ 0.28 substantially increased seed yield compared to the non-irrigated control (70% in 1990, and 160 % in 1991, Chapter 4). For 0H, the highest water-use efficiency treatment, seed crop water requirements were 280 mm in 1990 and 340 mm in 1991.

Table 3.1. Crop season length, change in soil water content, precipitation, applied water, seasonal and average daily estimated crop evapotranspiration (ET_c), pan evaporation, seasonal reference evapotranspiration (ET_r), and average seasonal water use (AWSU) during reproductive post-haying growth for six irrigation treatments for red clover seed at Hyslop Farm, Corvallis, OR, in 1990 and 1991.

Treat-	Crop season length	Change in soil water†	Precipi-tation	Applied water	Seasonal ET_c‡	Average daily ET_c§	Pan evapor-ation¶	Seasonal ET_r#	Average seasonal water use††
	d	mm							
Year 1990									
NS	86	38	56	446	540	6.27	499	412	1.31
HH	86	144	56	238	438	5.09	499	412	1.06
H0	74	185	35	134	354	4.78	462	383	0.92
0H	74	66	35	176	277	3.74	462	383	0.72
0L	66	173	12	64	249	3.77	433	355	0.70
C	66	206	12	0	218	3.31	433	355	0.61
Year 1991									
NS	95	44	69	354	467	4.92	475	452	1.03
HH	95	164	69	132	365	3.85	475	452	0.81
H0	82	208	46	42	296	3.62	459	389	0.76
0H	95	153	69	120	342	3.61	475	452	0.76
0L	82	174	46	57	277	3.38	459	389	0.71
C	82	242	46	0	288	3.51	459	389	0.74

†Data shown are soil water net change in total active profile (1.55 m deep).

‡Seasonal ET_c = water applied + precipitation + change in soil water content, during the crop season.

§Average daily ET_c = estimated crop evapotranspiration (ET_c) / crop season length (d).

¶Data from class A evaporation pan

#Seasonal reference evapotranspiration (ET_r) = pan coefficient x pan evaporation.

††Average seasonal water used (ASU) = seasonal ET_c / seasonal ET_r.

Table 3.2. Total above-ground phytomass and seed yield water use efficiencies of six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1991 and 1991.

Treat-ment	Total above-ground phytomass water use efficiency†			Seed yield water use efficiency†		
	1990	1991	Season contrast‡	1990	1991	Season contrast‡
	$kg\ ha^{-1}\ mm^{-1}\ ET_c$			$kg\ ha^{-1}\ mm^{-1}\ ET_c$		
NS	24.2d§	25.0b	ns	1.9c	1.8a	ns
HH	23.2d	26.1ab	*	2.0c	1.9a	ns
H0	27.1c	26.5ab	ns	2.9b	1.8a	**
0H	33.9a	27.4ab	***	3.5a	2.0a	***
0L	31.3b	28.5a	ns	3.4a	2.0a	**
C	31.6b	21.3c	***	2.6b	0.9b	***

ns,*,**,*** Not significant, significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

†Water use efficiency is expressed as the ratio of the component with estimated seasonal crop evapotranspiration (ET_c).

‡Probability that the water use efficiency means of the two years are different according to Student's t pairwise comparison.

§Within columns, means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 3.3. Fraction of available water used (FAWU) and crop water stress index (CWSI) at the time of water application for six red clover seed treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991.

	Year 1990				Year 1991			
	Post-haying		Peak-flowering		Post-haying		Peak-flowering	
Treatment	FAWU†	CWSI‡	FAWU	CWSI	FAWU	CWSI	FAWU	CWSI
	fraction of maximum amount							
NS§	0.12	0.01			0.05	-0.01		
HH	0.21	-¶	0.42	0.19	0.17	0.09	0.30	0.18
H0	0.21	-	0.38	0.19	0.14	0.09	0.23	0.17
0H	0.22	-	0.59	0.26	0.16	0.08	0.42	0.29
0L	0.21	-	0.53	0.26	0.16	0.10	0.40	0.29
C	0.21	-	0.58	0.27	0.23	0.09	0.48	0.29

†FAWU average standard error = 0.007

‡CWSI average standard error = 0.035

§Data shown are averages from all irrigation application times.

¶Data not taken.

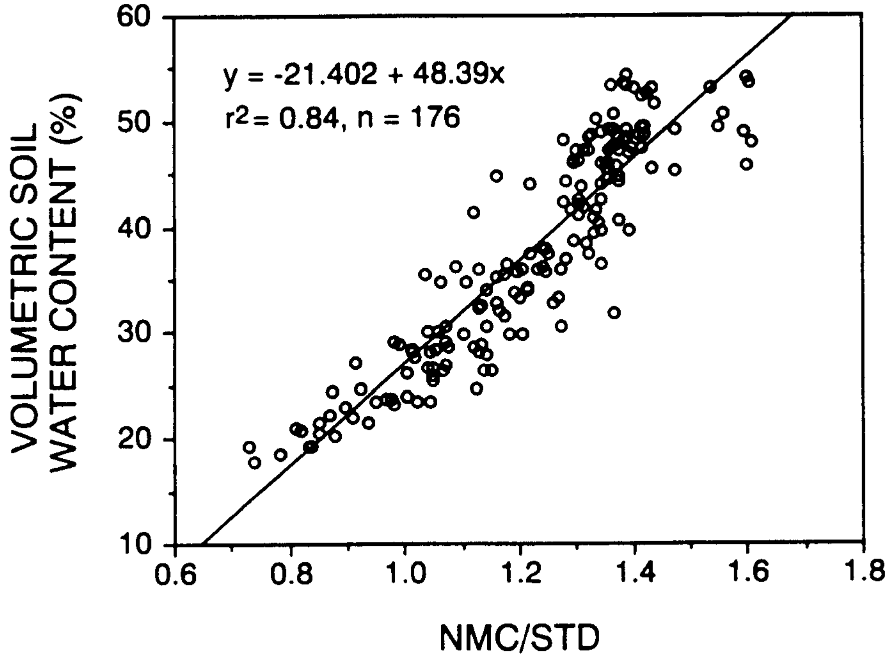

Fig. 3.1. Neutron probe calibration curve for red clover grown for seed at Hyslop Farm, Corvallis, OR in 1990 and 1991. NMC = neutron meter count, STD = probe standard count.

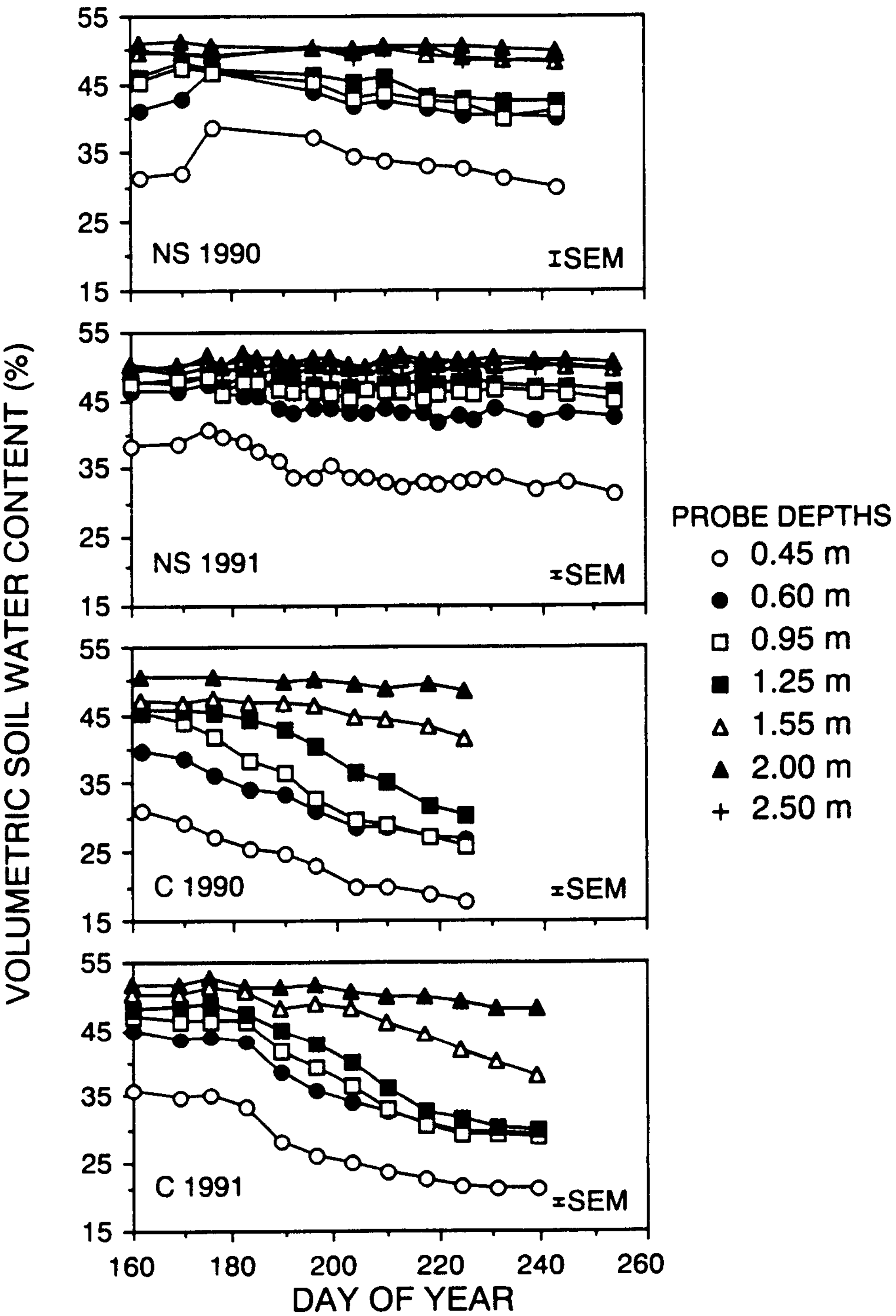

Fig. 3.2. Variation of volumetric soil water content with day of year at specific soil depths in non-stressed (NS) and non-irrigated (C) treatments for red clover seed at Hyslop Farm, Corvallis, OR in 1990 and 1991. Vertical bars indicate average standard error of the mean (SEM).

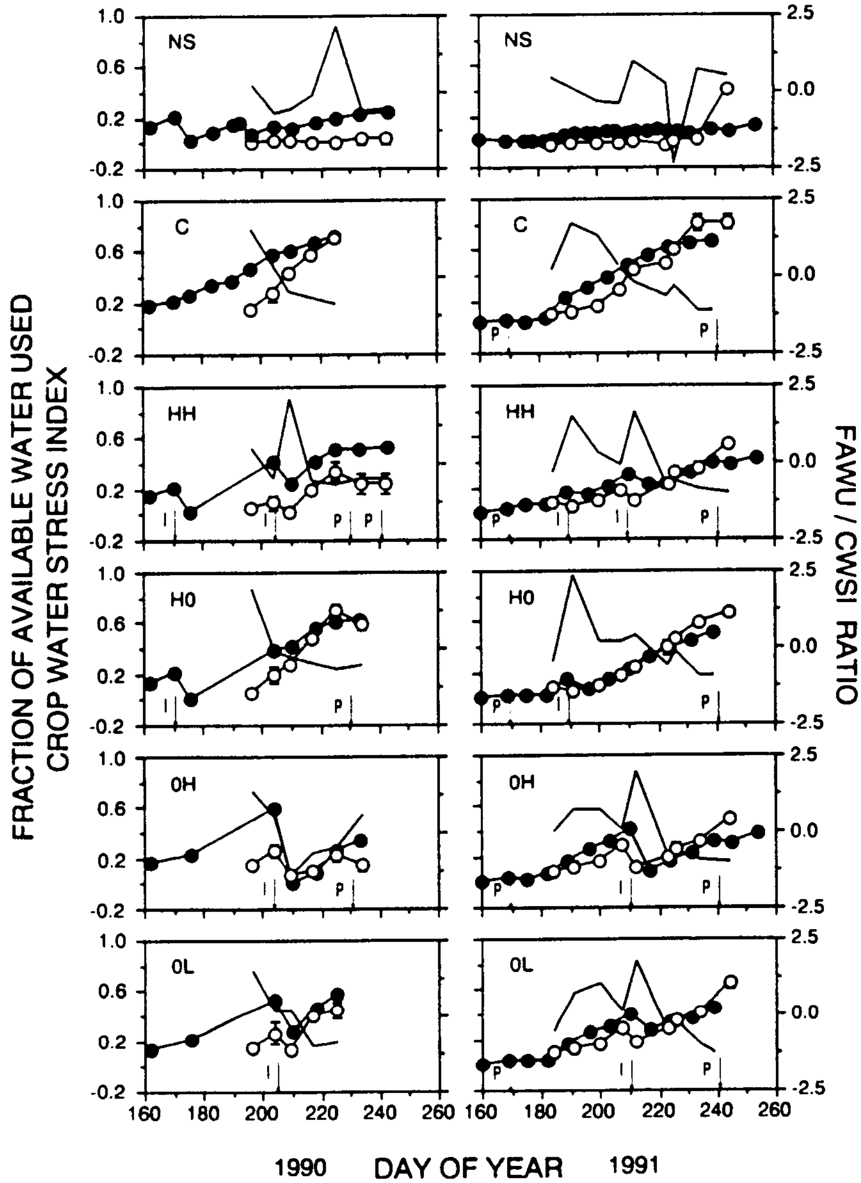

Fig. 3.3. Fraction of available water used (FAWU, •) and crop water stress index (CWSI, o) as functions of day of year for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991. Broken line graphs show FAWU/CWSI standardized ratio. Vertical bars indicate standard error of the mean. Arrows labelled I and P indicate irrigation application and precipitation (>10 mm) dates, respectively (not shown for treatment NS).

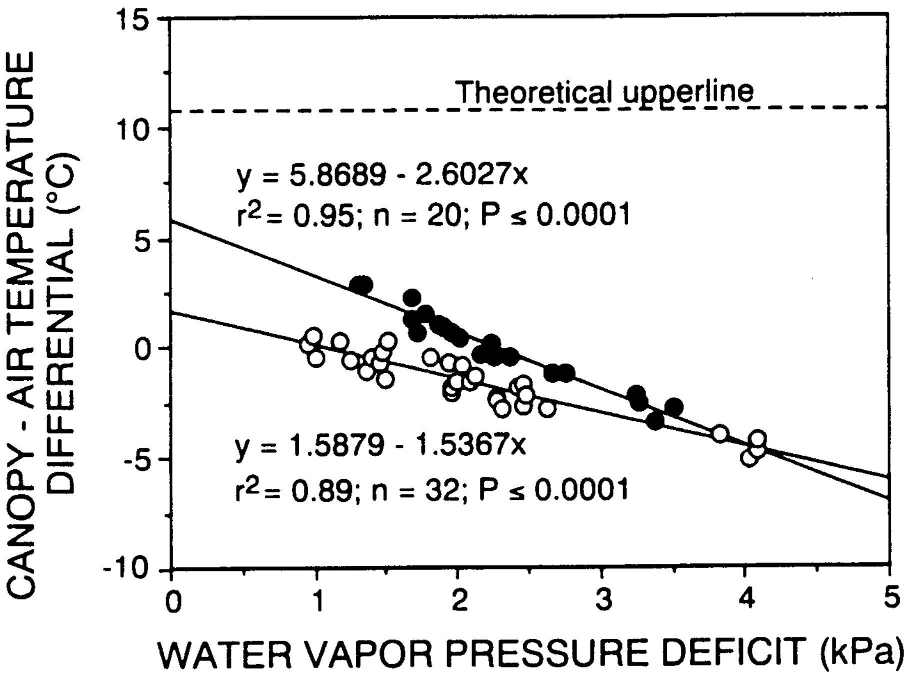

Fig. 3.4. Non-stressed baselines for canopy temperature minus air temperature versus water vapor pressure deficit relationship determined using data from non-stressed treatment (twice-weekly irrigation) before (o) and after (●) 50%of the red clover inflorescences were mature at Hyslop Farm, Corvallis, OR in 1990 and 1991.

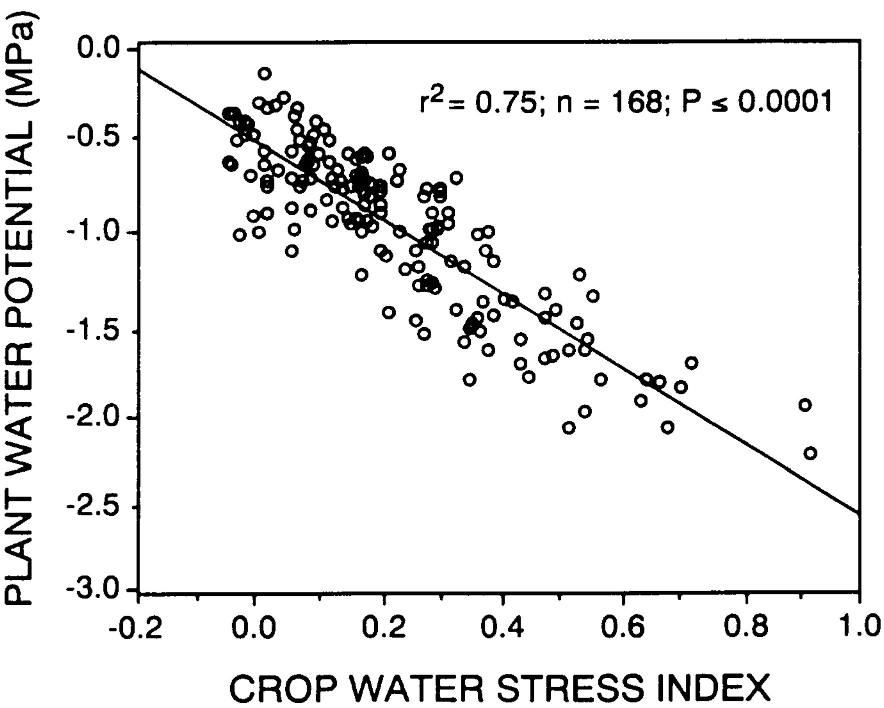

Fig. 3.5. Regression of plant water potential on crop water stress index from six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1991. Measurements were from days of year 191 to 234.

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CHAPTER 4

PLANT WATER STATUS EFFECTS ON RED CLOVER SEED YIELD AND YIELD COMPONENTS

ABSTRACT

Limited information on crop water stress response is available for red clover (*Trifolium pratense* L.) seed production. The purpose of this study was to quantify the effects of plant-water status on red clover inflorescence production, seed yield, and yield components. Five supplemental irrigation treatments were applied in 1990 and 1991 to first and second year red clover grown on a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll) near Corvallis, OR. In the non-stressed treatment, the soil water content was brought to field capacity by twice weekly replacement of water used since the prior application. Two treatments had post-haying water replacement to 100% of field capacity, one of which again had the soil profile replenished to 100% field capacity at peak flowering time. Two treatments received single applications of water to soil water contents of 50 and 100% of field capacity at peak flowering. A non-irrigated control was also maintained. Increased plant water stress reduced the duration of the season-long bud and flower production, stem length, potential floral capacity (PFC), and seed yield (SY). Application of water soon after haying contributed to increased total above-ground phytomass (TAGP) but benefited seed yield less than watering at peak flowering. Root deterioration from root and crown rot disease complex reduced second-year SY. Root rot index (RRI) increased with increasing levels of plant water stress, indicating that supplemental water applications reduced root rot severity. The reduction in SY from increasing plant water stress was primarily caused by a decrease in floral fertility, and less conclusively by reductions in inflorescence number per unit area. One irrigation to fill the soil active profile during peak flowering provided adequate water to maintain efficient seed production. Under this optimum water management treatment, potential seed yield (SY_{pot}) utilization and seed yield were substantially increased in relation to the non-irrigated control.

INTRODUCTION

Limited information is available for red clover water management and crop water stress response. Highest seed yields in the western U.S. were reported to be obtained by frequent irrigations which kept plants growing vigorously throughout the vegetative and seed setting periods (Fergus and Holowell, 1960; Rincker and Rampton, 1985). In New Zealand, however, irrigation after the removal of grazing pressure (closing) was reported to increase vegetative growth, reduce flowering, and was considered detrimental to seed yield (Clifford and Anderson, 1980). Irrigation management can substantially increase seed yields in some forage legumes by increasing inflorescence density and floral fertility such as in white clover (Clifford, 1987), or increasing floral fertility as in alfalfa (Cohen et al., 1972; Steiner et al., 1992). To the best of our knowledge, the effect of water management on seed yield components for red clover has not been documented.

The purpose of this research was to quantify the effects of plant water status on red clover inflorescence production and development, and seed yield and yield components in western Oregon.

Abbreviations: SY_{act}, actual seed yield; CWSI, crop water stress index; ET_c, estimated crop evapotranspiration; HI, harvest index; PFC, potential floral capacity; SY_{pot}, potential seed yield; RRI, root rot index; SBF, seed bearing flowers; SY, harvested seed yield; TAGP, total above-ground phytomass.

MATERIALS AND METHODS

The experiment was conducted as a randomized complete block design with four replications and six treatments for two years at the Hyslop Research Farm near Corvallis, OR. The soil was a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll).

Each experimental unit (EU) was 15 rows, 10 m long (45 m^2). Each EU was surrounded by a furrow with dikes to prevent lateral surface water movement. One-meter wide alleyways at the ends of EUs were also diked. The experimental area was fumigated with methyl bromide (360 kg ha^{-1}) prior to seed bed preparation to control weeds. Medium red clover (*Trifolium pratense* L.) 'Kenland' (Hollowell, 1951) was sown 14 September 1989 in single rows, 0.3 m apart, at a rate of 1.7 kg ha^{-1}. One 25-mm sprinkler irrigation was applied after seeding to establish the crop. The plots were harvested for seed during August and September in 1990 and 1991.

Crop culture followed common commercial practices for western Oregon. All plots were hayed in spring (day of year 162 in 1990 and 160 in 1991). Haying at early-flowering is an operation that synchronizes blooming with warm weather when insect pollinators are fully active and also helps control some weeds and several insect pests. Aphids (*Nearctaphis bakeri* (Cowen)) and lygus bugs (*Lygus* spp.) were controlled with 0,0, dimethyl S-[2-ethylsulfinyl)-ethyl] phosphorothioate applied at bud stage of development at a rate of 1 kg ha^{-1}. Annual grasses and broadleaf weeds as well as volunteer clover seedlings were controlled in winter 1991 with diuron at a rate of 3.2 kg ha^{-1}. Four honey bee (*Apis mellifera* L.) hives were placed adjacent to the experimental area at the beginning of bloom time. Honey bee and naturally occurring bumble bee (*Bombus sp.*) activity was sufficient for adequate pollination.

All EUs had similar soil-water contents at the beginning of each cropping period because rainfall during the winter maintained soil water at or above field capacity. A surface trickle irrigation system consisting of a mesh filter, ball valve, residential water flow meter, volumetric controller, and pressure regulator, distributed water to each EU. Within each EU, water was delivered through five plastic trickle lines 0.9 m apart and placed parallel to the planted rows. The trickle lines were fitted with in-line turbulent-flow emitters spaced 0.9 m apart that delivered 4 L of water h^{-1}. Water was distributed to all four replicates of each treatment at the same time.

Five supplemental water application treatments were applied during the cropping period from haying to seed harvest. The treatments were: NS) non-stressed, the soil was brought to field capacity by twice-weekly replacement of the soil water used since the last application until 3 weeks before seed harvest; HH) two irrigations to refill the soil profile to 100% of field capacity, one when the soil water depletion was 20% after haying and the second at peak flowering; H0) single water replacement to 100% of field capacity when the soil water depletion was 20% after haying; 0H) single water replacement to 100% of field capacity at peak flowering; and 0L) single water replacement to 50% of field capacity at peak flowering. A non-irrigated control (C) was also maintained. Peak flowering is defined as the time when the crop displayed a maximum number of inflorescences in anthesis as determined by weekly observations.

Seasonal estimated crop evapotranspiration (ET_c) was determined by the summation of applied water, precipitation, and the change in soil water content determined by neutron probe measurements (Chapter 3). Plant water status was monitored from the beginning of flowering until harvest using infrared thermometry techniques and crop water stress index (CWSI) values were determined (Chapter 3). Average seasonal CWSI was calculated as the mean of weekly CWSI values within each year.

In 1990, five floral buds per EU were tagged at DOYs 183 and 196 during the flowering period in treatments NS, 0H and C. A floral maturity index based on ten phenological stages was used to study the time-course inflorescence development. In both years three 0.1 m^2 random samples per EU were counted weekly to determine inflorescence production for each treatment. Plant height was also measured. The phenological stages used were: 1) floral bud: youngest floral meristem (visible bud) to fully expanded head with less than 50% opened florets, 2) flowering head: majority of florets opened to less than 50 % desiccated florets, and 3) seed bearing flowers (SBF): majority of florets desiccated to dry seed. PFC was calculated by the cumulative sum of floral buds through the growing season. PFC was much easier to measure and consequently was more consistent among treatments and years than SBF. As a result, PFC instead of SBF was considered as the inflorescence density seed yield component.

An 8-m section of the three center rows (7.2 m^2) of each EU was harvested with a gas-powered mower in early-morning when 80% of the florets were dry and able to shatter. The plant material was gathered by hand and put in burlap bags and dried at 32°C for 3 d. Above-ground phytomass was weighed and the seeds threshed, cleaned, and weighed to obtain clean SY. Seeds were stored at 10 ºC and 35 % RH. Harvest index (HI) was calculated dividing SY by TAGP.

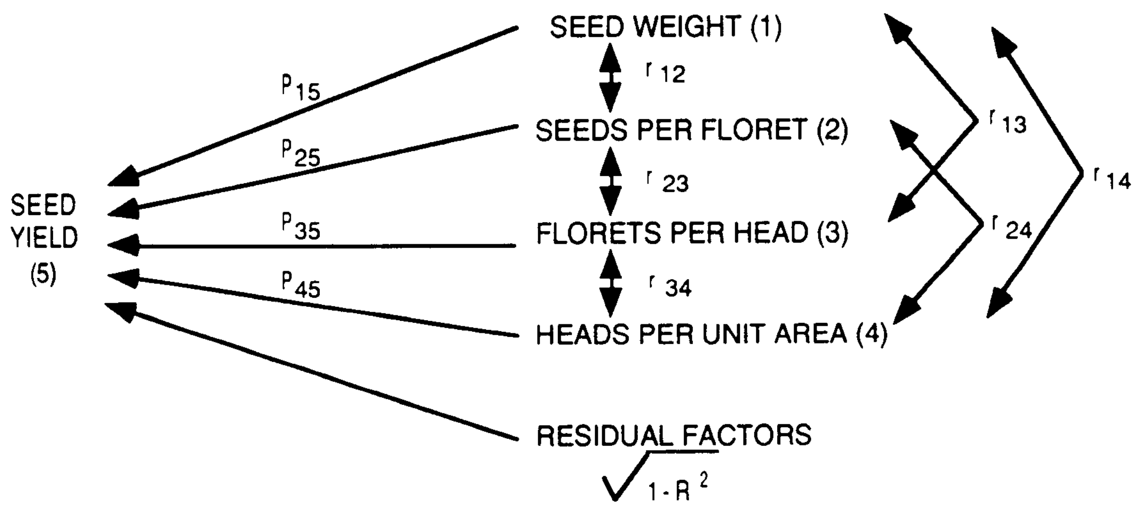
At harvest, three 0.1 m^2 random samples per EU were taken to determine the number of florets per inflorescence (florets from 10 random flower heads per sample) and the number of seeds per pod (50 random florets per sample).

Forage legume seed yield potential is defined as the number of ovules (or potential seed sites) per unit ground area at anthesis (Lorenzetti, 1981). SY_{pot} was calculated as the product of the number of inflorescences per unit area, number of florets per inflorescence, unit seed weight, and the genetically determined two seed sites per pod. Actual seed yield (SY_{act}) was calculated using the measured values of all the yield components.

Immediately after second year seed harvest, 20 random roots were taken from each EU and root damage and root diameter at crown level determined. A modification of the index proposed by Gagnon (1979) was used as a RRI. RRI was based on a 0 to 5 scale: 0) completely healthy root tissues, 1) presence of superficial light-brown lesions affecting less than 20% of the taproot, 2) presence of dark-brown lesions affecting 20 to 40% of the taproot, 3) presence of extended and profound dark-brown lesions affecting 40 to 60% of the taproot, 4) presence of extended and profound dark-brown lesions affecting 60 to 80% of the taproot, and 5) more than 80% of the taproot tissue decayed. Plant density at that time was estimated based on percent ground coverage of ten 0.5 m^2 observations per EU.

Seed moisture content was determined from two random 5-g samples per EU dried at 130 ºC for 1 h (Grabe, 1989). Mean seed weight per EU was determined from three samples of 200 seeds. For the germination test, three replicates of 50 seeds were placed in chambers at 19 °C for 7 days. At the end of the test, healthy seedlings were counted to determine percent germination (Association of Official Seed Analysts, 1978). Seed vigor was estimated with 20 seeds per EU placed on a line drawn 35 mm from the upper end of a moist blotter inside a polystyrene box. The boxes were held at an angle of 70° from the horizontal in a chamber at a constant temperature of 19 °C. Germinated seeds were recorded daily until day 7 to calculate mean germination time (mean germination time = $\Sigma X_i T_i / \Sigma X_i$, where X_i = number of newly germinated seeds at time T_i). On the final count, radicle and plumule lengths of all seedlings with visible radicles were measured with a 10 x 10 cm, 2 mm^2 grid, plastic transparency (Oliva et al., 1987). Before testing, seeds were scarified on sandpaper, under a 50 PSI air flow for 20 s.

Seed yield components were subjected to path-coefficient analysis across the six irrigation treatments to partition the correlation coefficient into components of direct and indirect effects (Dewey and Lu, 1959). Results were then related to the association between irrigation treatments and components of seed yield to determine the effects of plant water stress on the relative importance of each seed yield component. The five variables included in the path-coefficient analysis and the direction of their causal relationship are represented by:



Double-arrowed lines indicate mutual associations which are measured by correlation coefficients (r_{ij}), and single-arrowed lines represent direct effects measured by path coefficients (P_{ij}).

All variables were tested by analysis of variance. Simple linear regression analysis was used to relate TAGP and seasonal ET_c; TAGP, SY, and

RRI with average seasonal CWSI; and TAGP and root diameter with RRI. Standard errors of the mean were calculated for SBF, PFC, CWSI, and main stem length measurements. Student's t pairwise comparisons were used to contrast SY, TAGP, and HI means between years 1990 and 1991. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Crop water stress and irrigation treatment relationships. A single value of average seasonal CWSI represented the mean plant water status for each irrigation treatment during flowering and seed development in 1990 and 1991 (Table 4.1). These values were similar within treatments for both years so one value describes plant water stress level within each treatment. NS, HH, and 0H (soil at field capacity at peak flowering), and H0, 0L, and C (soil below field capacity at peak flowering) were grouped as low and high water stress treatments, respectively. NS, HH, and H0 were considered as early irrigated treatments and 0H and 0L as late irrigated treatments.

Inflorescence production and development. The time of inflorescence initiation was the same for all treatments because of the general low levels of plant water stress at the beginning of the flowering period (Fig. 4.1). Generally, increasing levels of water stress during flowering reduced the time from initiation to maturity for individual inflorescences. In the non-stressed treatment, inflorescences required about 35 d to mature while non-irrigated inflorescences matured 7 d earlier (data not shown). The duration of season-long flower production was reduced as the water stress conditions increased (Fig 4.2.). Treatment C flowered for 43 and 60 days compared to 65 and >73 days for treatment NS in 1990 and 1991, respectively. In 1990, 15% of the available soil water had been depleted at haying, while at that time in 1991 the soil was still at field capacity (Chapter 3). As a consequence, those treatments in 1990 without irrigation water soon after haying (0H, 0L, and C) had peak flowering times 2 wk earlier than the less-water stressed treatments (NS and HH), while all treatments had the same peak flowering time in 1991 (Fig 4.2).

Since the inflorescence production period was shorter for high- than low-stress treatments, the rate of SBF production increased with increasing water stress levels (Fig. 4.1). Low water stress conditions, especially during early stages of reproductive development, increased PFC by increasing stem length (Fig. 4.2) and duration of bud production (Fig. 4.1). PFC was similarly correlated with stem length both years (r = 0.96). Application of water after haying favored excessive stem elongation that led to lodging in NS, HH and H0 in 1990. Subsequently, some flowers either aborted or rotted under the canopy, especially in those treatments that received irrigation water after lodging (NS, HH). In 1991, longer stems and resulting larger plants increased PFC in all treatments compared to 1990. Resulting plant lodging in all treatments prevented higher SBF production from being realized (Fig. 4.1). Therefore, SBF values were the same for all treatments both years (data not shown). Different levels of plant lodging within treatments and flower loss under the canopy account for the differences between SBF and PFC.

Seed yield. SYs were greater in 1990 than in 1991 for all treatments (Table 4.1). The non-irrigated control (C) yielded less than all other treatments in both years. Highest water use efficiencies and optimum SYs were obtained from a single application of water (0H) which filled the soil profile at peak flowering (Chapter 3). Within each year, SY increased with increasing seasonal ET_c to approximately 275 mm in 1990 and 350 mm in 1991 (Fig. 4.3). Above these ET_c values, there was little response to increasing ET_C. Non-stressed (NS) and twice-irrigated (HH) treatments showed luxury consumption of water with minimal gains in SYs.

Unlike SY, TAGP increased linearly with increasing ET_c (Fig. 4.3). This indicated that there was no luxury consumption of water in relation to TAGP production. Consequently, SY increased with TAGP asymptotically (data not shown). All crop water use was related to TAGP increases, while only lesser amounts of water were associated with maxima in SYs. However, when plant water status during the flowering period (average seasonal CWSI) instead of the amount of water used during the entire cropping season (seasonal ET_c) was considered, both SY and TAGP decreased linearly with increasing average seasonal CWSI (Fig. 4.4). Application of water after haying (NS, HH, H0) contributed to increasing TAGP but generally did not benefit SY. Since available soil water was already high at haying (> 80% available soil water), water applied at peak flowering rather than after haying more efficiently affected SY and resulted in higher water use efficiency values (Chapter 3).

Lower second-year SYs for similar average seasonal CWSI values indicated that some factors other than water stress alone limited seed production. Average second-year TAGP production was only 8 % lower than that of the first year, and was not related to the SY decrease (Table 4.1). Larger plants in 1991 than in 1990 increased PFC but seed set and yield was reduced (Table 4.2). Thus, HI values were lower in 1991 than in 1990 for all treatments (Table 4.1). In 1990, HI was affected by water stress levels and associated different lodging among treatments. Lodging in 1991 was similar for all treatments and then HI values were more comparable across the different water stress levels. Considering the effects of lodging on NS and HH in 1990, HI was similar both years for all watered treatments but decreased markedly in the non-irrigated control (Table 4.1).

Root deterioration from root and crown rot disease complex caused primarily by *Fusarium* spp. was observed to have affected all plants after the 1991 seed crop was harvested. This disease complex caused primarily by *Fusarium* spp. is consider to be a limiting factor for the continued productivity of forage legume stands and the main reason why red clover cannot produce a satisfactory seed yield beyond the year after seeding (Leath, 1985).

RRI increased with increasing levels of average seasonal CWSI, indicating that supplemental water applications reduced root rot (Fig. 4.4). This agrees with the findings that *Fusarium* root rot is favored by low soil water content (Nan et al., 1991). The root rot-limiting effects were more severe on reproductive than vegetative yield. TAGP increased about 100% from the most to the least root-damaged treatments, while seed yield increased more than 200% under the same conditions. Root diameter decreased as RRI increased (Fig. 4.5). These results confirm the suggestion that adequate conditions for root growth lessen the damage caused by root rot (Nan et al., 1991). Since root health was only evaluated at the end of the second-year, the epidemiology of the disease for the duration of the experiment is not known. It is likely that root rot triggered by plant water stress conditions decreased photosynthate translocations to the primary root, and thus reduced the size and function of the taproot. Consequently, root breakdown after full PFC may have caused the decrease in floral fertility and subsequent lower SYs in 1991 compared to 1990. Although persistence ultimately influences stand depletion (Kendall and Stringer, 1985), no relationship between either RRI or CWSI with plant density was found after the second-year seed crop. However, plant losses were higher for the non-watered control treatment compared to all watered treatments (data not shown).

Components of seed yield and seed yield potential. All seed yield components were affected by water treatments in both years (Table 4.2). Inflorescence density was greater in all irrigated treatments, especially those watered early in the season (NS, HH ,H0). There were more inflorescences per unit area in 1991 than in 1990. In 1990, more florets per inflorescence were produced in late- and non-irrigated treatments (0H, 0L, C). In 1991, there was no predictable relationship between treatments and number of florets per inflorescence. Floral fertility (seeds per floret) was lowest in C, intermediate in 0L, and highest in NS, HH, H0, and 0H, both years. All treatments had reduced floral fertility in 1991 compared to the previous year. Seed weight was lower both years for those treatments with increased levels of plant water stress during seed development (H0, 0L, C). Higher seed weight for all treatments in 1991 than in 1990 may have been the result of reduced sink size that contributed to increased photosynthate allocation to the fewer number of developing seeds (Table 4.2).

Except for florets per inflorescence in the second year, all yield components were associated with SY in 1990 and 1991 (Table 4.3). However, correlation coefficients alone do not reveal how individual yield components affect SY and the extent of collinearity. Path-coefficient analysis provides an effective tool to separate direct and indirect causes of association and measures the relative importance of each seed yield component across the irrigation treatments.

Seeds per floret was the most influential yield component affecting SY both directly and indirectly both years (Table 4.3). Seed weight and florets per inflorescence affected seed yield directly and indirectly in 1990, but not in 1991. SY in 1990 was not affected by inflorescences per unit area. However, since plant lodging and subsequent loss of flowers did not affect all treatments similarly, the results were not conclusive. Similar plant lodging in all treatments in 1991 showed that SY was affected only by the interaction between seeds per floret and inflorescences per unit area. In both years there was a clear trend of yield compensation between florets per inflorescence and the rest of the yield components. Overall, the reduction in SY from increasing plant water stress was primarily caused by the decrease in floral fertility, and less conclusively by reduction in the number of inflorescences per unit area. None of the treatments achieved maximum floral fertility, substantiating the suggestion that there is a level of seed set above which the plant cannot support all fertilized ovules through maturity (Clifford and Scott, 1989).

Seed weight decreased as average seasonal CWSI increased both years, especially at high levels of plant water stress (treatment C, data not shown). However, variations in seed weight did not affect seed quality. Average treatment mean time to germination was 2.3 d $\pm$ 0.2 in 1990 and 2.1 d $\pm$ 0.1 in 1991. Total 7-d seedling germination length was 60 mm $\pm$ 2 and 55 mm $\pm$ 3 in 1990 and 1991, respectively. Percent germination for all treatments after scarification was 99.7% $\pm$ 0.2 in 1990, and 98.4% $\pm$ 0.8 in 1991.

SY_{pot} was calculated using a theoretical two seed sites per floret. Consequently, variation in SY_{pot} among treatments was due to differences in inflorescences per unit area, florets per inflorescence, and seed weight (Table 4.2). Early application of water (NS, HH, and H0) tended to increased plant size and PFC. However, only NS had consistently high SY_{pot} both years while the rest of the watered treatments tended to have similar SY_{pot} because of compensatory relationship among yield components. The differences between

SY_{pot} and SY_{act} were due to reduced actual floral fertility. As a consequence, SY_{act} as a percentage of SY_{pot} decreased in the high water stressed treatments (0L and C) both years. The reductions in SY_{act} compared to SY_{pot} for all treatments (50 to 72%) were generally lower than the 75% value cited for red clover by Lorenzetti (1981). SY was lower than the theoretical SY_{act} in all cases, but especially in 1991 (Table 4.2). This may have been the result of inflorescences lost under the canopy during the season, and seed losses during harvest, threshing and cleaning. SY as a percentage of SY_{pot} was higher than the 20% value cited by Lorenzetti (1981) in 1990, but about the same in 1991 for all low water stress treatments (22 to 30% and 19 to 20%, respectively). Both years the non-irrigated control had the lowest values of SY as a percentage of SY_{pot}.

SY_{pot} was very high, even when considering the least-productive treatment (2,600 kg ha-1 for treatment C in 1990; Table 4.2). These results substantiate the suggestion of Hampton (1991) that SY reductions are a consequence of poor SY_{pot} utilization rather than low SY_{pot} establishment. SY_{pot} establishment depends mainly on the number of inflorescences per unit area and the number of florets per inflorescence at anthesis. SY_{pot} utilization is determined by events at and after anthesis, such as pollination, fertilization and seed growth. Thus, SY_{pot} utilization depends on the number of seeds per floret and unit seed weight (Hampton, 1990).

In NS, SY was only 30 and 19% of the SY_{pot} in 1990 and 1991, respectively. Water stress effects cannot explain all differences between SY_{pot} and SY, but proper water management can substantially improve SY and enhance SY_{pot} utilization. Increasing SY from 569 kg ha-1 (C) to 981 kg ha-1 (0H) in 1990 and from 265 kg ha-1 (C) to 691 kg ha-1 (0H) in 1991 represented improvements of 72 - 160% in SY and 13 - 10% in SY_{pot} utilization, respectively.

CONCLUSIONS

Supplementary irrigation improves red clover seed yield under western Oregon climatic conditions. Plant water stress levels above an average seasonal CWSI of 0.4 were highly detrimental to seed yield. Floral fertility was the yield component most negatively affected by water stress. Root rot during the second year of seed production was aggravated by plant water stress. Unlike other forage legume seed crops (Clifford, 1987; Steiner et al., 1992, Chapter 4), red clover seed production was maximized under the same agronomic conditions as green matter production (e.g. non stress conditions of treatment NS). A non-stressed irrigation scheme would be impractical if not impossible and would greatly increase plant lodging. A single irrigation filling the soil active profile during peak flowering (CWSI $\approx$ 0.28, Chapter 3) maintained low levels of plant water stress, optimized seed yield and improved potential seed yield utilization.

Table 4.1. Seed yield (SY), total above-ground phytomass (TAGP), harvest index (HI), average seasonal crop water stress index (CWSI), and seasonal estimated crop evapotranspiration (ET_c) for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991. Season contrasts for seed yield, total above-ground phytomass and harvest index are shown.

	1990					1991					Season contrast		
Treatment	Seed yield	TAGP	Harvest index	Average seasonal CWSI	Seasonal ET_c	Seed yield	TAGP	Harvest index	Average seasonal CWSI	Seasonal ET_c	SY	TAGP	HI
	-- 1000-kg ha^{-1} --				mm	-- 1000-kg ha^{-1} --				mm			
NS	1.05a†	13.03a	8.1b	0.02	539	0.83a	11.68a	7.1a	0.04	467	**	*	*
HH	0.88b	10.15b	8.7b	0.18	438	0.69b	9.52b	7.2a	0.22	365	*	ns	**
H0	1.02a	9.59b	10.7a	0.32	354	0.53c	7.86c	6.7a	0.31	297	**	*	***
0H	0.98ab	9.37b	10.5a	0.16	277	0.69b	9.38b	7.4a	0.23	343	***	ns	***
0L	0.85b	7.78c	10.9a	0.28	249	0.55c	7.90c	6.9a	0.29	277	*	ns	**
C	0.57c	6.90d	8.3b	0.42	218	0.27d	6.14d	4.3b	0.43	288	***	ns	**

ns, *,**,***Not significant, significant at 0.05, 0.01, 0.001 probability levels, respectively.

†Within columns, means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 4.2. Seed yield components for six red clover irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991.

Treatments	Inflorescences	Florets per inflorescence	Seeds per floret	Seed weight	Seed yield: Potential†	Seed yield: Actual‡	Seed yield: Harvested§	Seed yield as percentage of potential: Actual	Seed yield as percentage of potential: Harvested
	no. m^{-2}			mg	$g\ m^{-2}$			%	
Year 1990									
NS	934a¶	103c	0.99a	1.79a	344a	171a	105a	50a	30a
HH	704b	103c	0.90b	1.79a	260b	117bc	88b	45b	34a
H0	725b	123b	0.89b	1.74b	310a	138b	102a	45b	33a
0H	584c	133ab	0.89b	1.80a	280b	124b	98ab	45b	35a
0L	579c	137ab	0.66c	1.77ab	281b	93c	85b	33c	30a
C	577c	142a	0.56d	1.59c	261b	73c	57c	28c	22b
Year 1991									
NS	944a	123ab	0.76ab	1.90ab	441a	168a	83a	38ab	19ab
HH	819b	108bc	0.80a	1.92a	340bc	136b	69b	40a	20a
H0	815b	101c	0.73ab	1.86bc	306c	112cd	53c	37ab	17ab
0H	767bc	127a	0.76ab	1.91a	372b	141b	69b	38ab	19ab
0L	796b	124a	0.70bc	1.85bc	365b	128bc	55c	35bc	15b
C	698c	119ab	0.63c	1.83c	304c	96d	27d	32c	9c

†Potential seed yield = inflorescences per m^2 x florets per inflorescence x seed weight x 2 (potential seed sites per floret).

‡Estimated using actual number of seeds per floret.

§Seed harvested and cleaned.

¶For each year, within-column means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 4.3. Path coefficient analysis across all red clover seed irrigation treatments at Hylsop Farm, Corvallis, OR in 1990 and 1991.

Pathway	1990	1991
Seed weight *vs.* seed yield		
Direct effect, P_{15}	0.35*	0.14ns
Indirect effects		
via seeds per floret, $r_{12}P_{25}$	0.43	0.28
via florets per inflorescence, $r_{13}P_{35}$	-0.19	0.03
via inflorescences per unit area, $r_{14}P_{45}$	0.07	0.12
Correlation, r_{15}	0.66**	0.57**
Seeds per floret *vs.* seed yield		
Direct effect, P_{25}	0.65**	0.45*
Indirect effects		
via seed weight, $r_{12}P_{15}$	0.23	0.09
via florets per inflorescence, $r_{23}P_{35}$	-0.25	-0.09
via inflorescences per unit area, $r_{24}P_{45}$	0.18	0.21
Correlation, r_{25}	0.82**	0.66**
Florets per inflorescence *vs.* seed yield		
Direct effect, P_{35}	0.36*	0.26ns
Indirect effects		
via seed weight, $r_{13}P_{15}$	-0.19	0.02
via seeds per floret, $r_{23}P_{25}$	-0.45	-0.15
via inflorescences per unit area, $r_{34}P_{45}$	-0.18	-0.03
Correlation, r_{35}	-0.46*	0.10
Inflorescences per unit area *vs.* seed yield		
Direct effect, P_{45}	0.28ns	0.42**
Indirect effects		
via seed weight, $r_{14}P_{15}$	0.09	0.04
via seeds per floret, $r_{24}P_{25}$	0.43	0.23
via florets per inflorescence, $r_{34}P_{35}$	-0.24	-0.02
Correlation, r_{45}	0.56**	0.67**

ns,*,**Not significant, significant at the 0.05 and 0.01 probability levels, respectively.

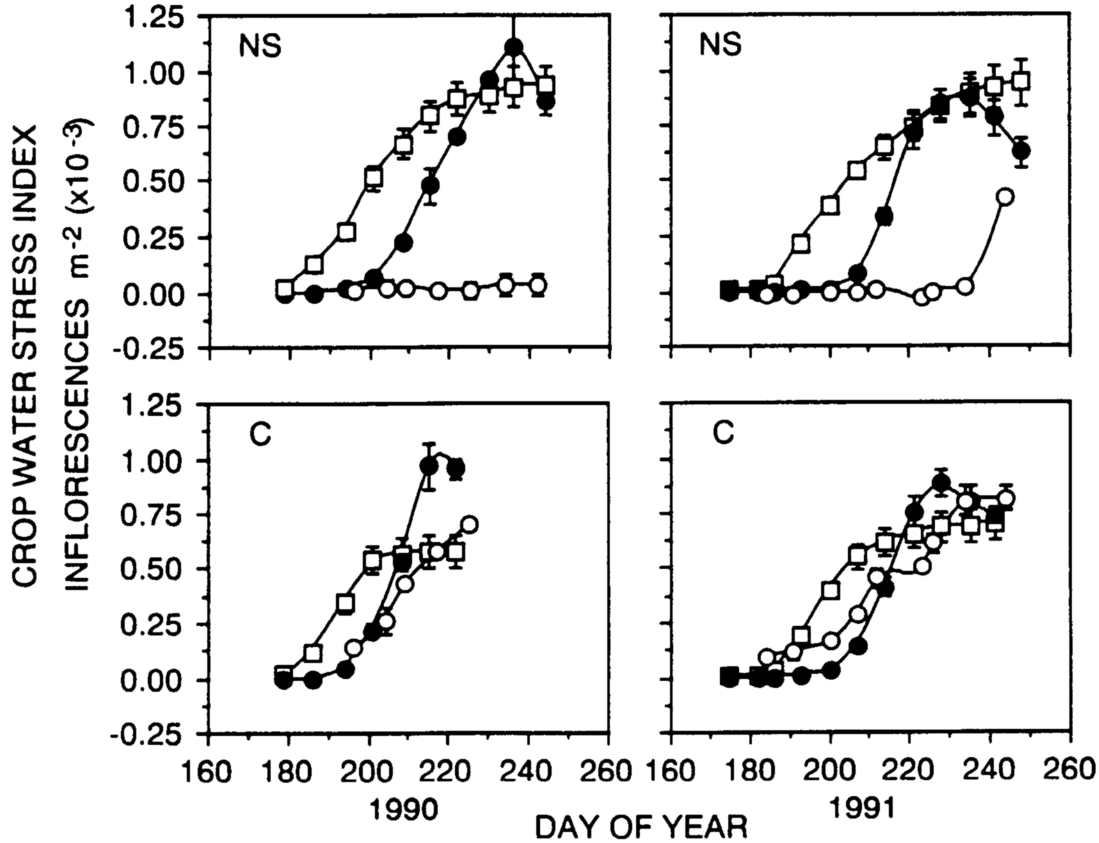


Fig. 4.1. Seed bearing flowers (●), potential floral capacity (□), and crop water stress index (○) as function of day of year in non-stressed (NS) and non-irrigated (C) treatments for red clover grown for seed at Hyslop Farm, Corvallis, OR in 1990 and 1991. Vertical bars indicate standard error of the mean.

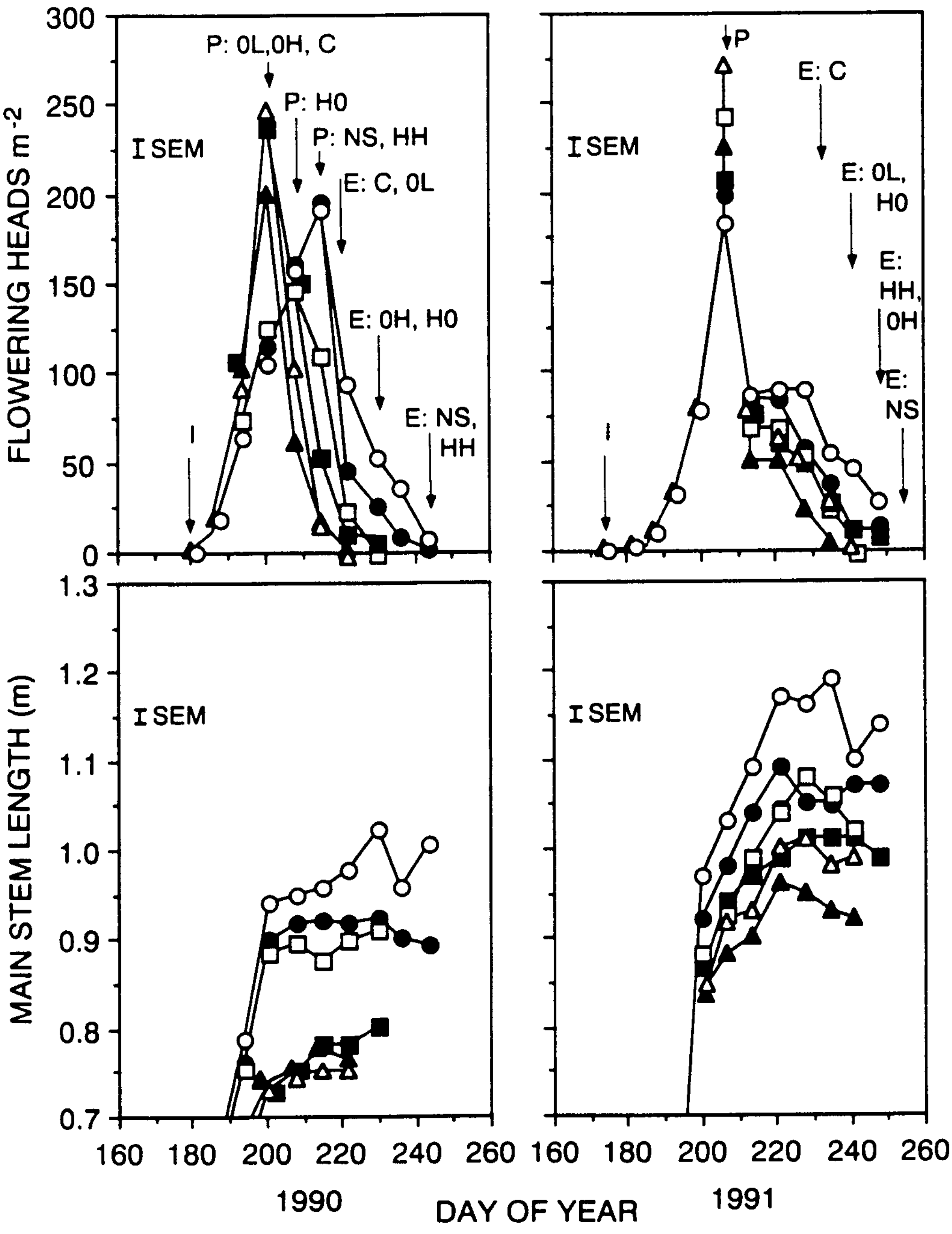


Fig. 4.2. Number of flowering heads and main plant stem length as function of day of year for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991. ○NS, ●HH, □H0, ■0H, △0L, and ▲C. Arrows labelled with I, P and E indicate flower initiation, peak flowering and end of flowering dates for the treatments indicated, respectively. When no treatments are given after a phenological label, all treatments had the same date for that phenological stage. Vertical bars indicate average standard error of the mean (SEM).

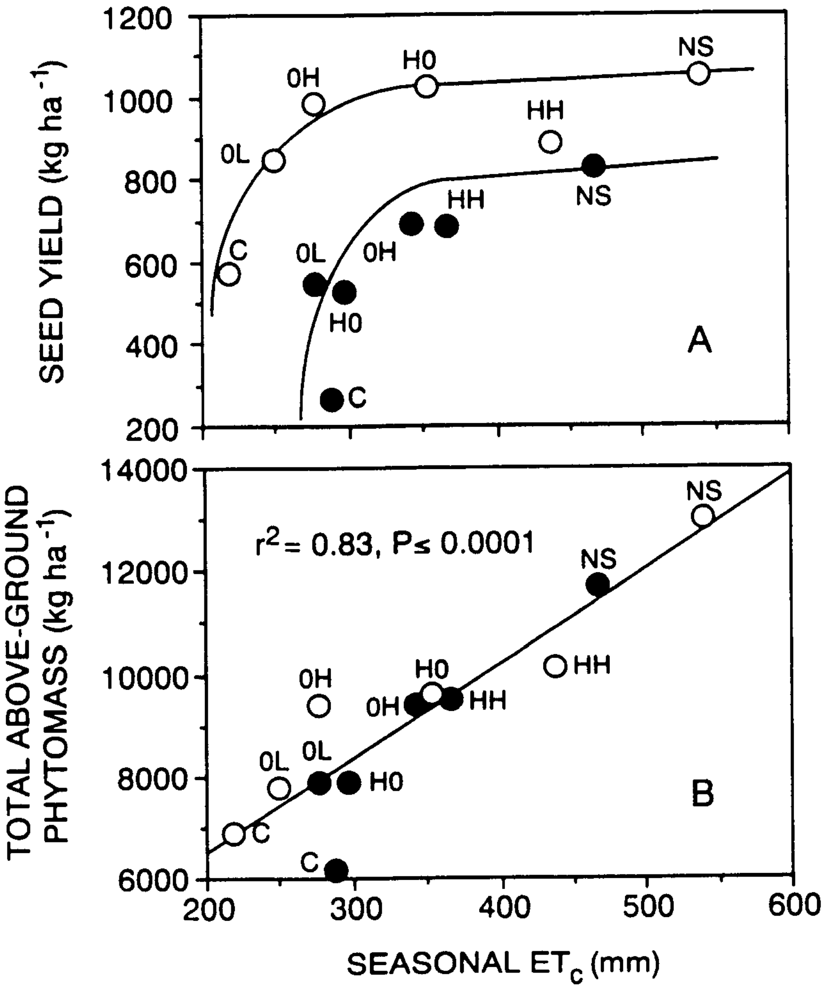


Fig. 4.3. (A) Seed yield (SY) and (B) total above-ground phytomass (TAGP) as function of seasonal estimated crop evapotranspiration (ET_c) for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (○) and 1991 (●). Hand-drawn lines in A show the trend to initially high yield responses as ET_c value increases, and thresholds where no significant yield increments are expected. Treatments are indicated beside every data point.

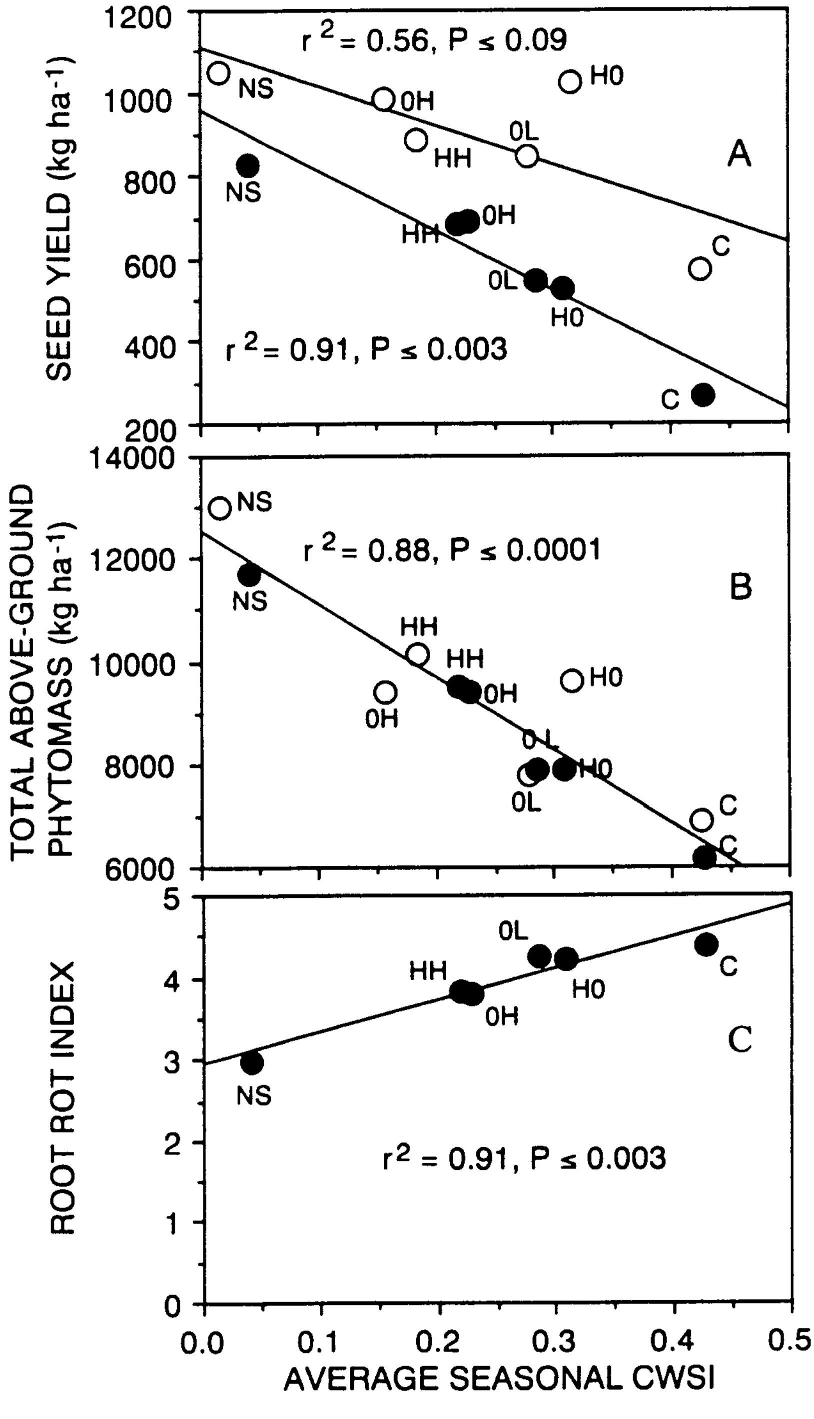


Fig. 4.4. (A) Seed yield (SY), (B) total above-ground phytomass (TAGP) and (C) root rot index (RRI) as function of average seasonal crop water stress index (CWSI) for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (○) and 1991 (●). RRI is based on a 0 to 5 scale, where 0 = no damage and 5 = > 80% damage. Treatments are indicated beside every data point.

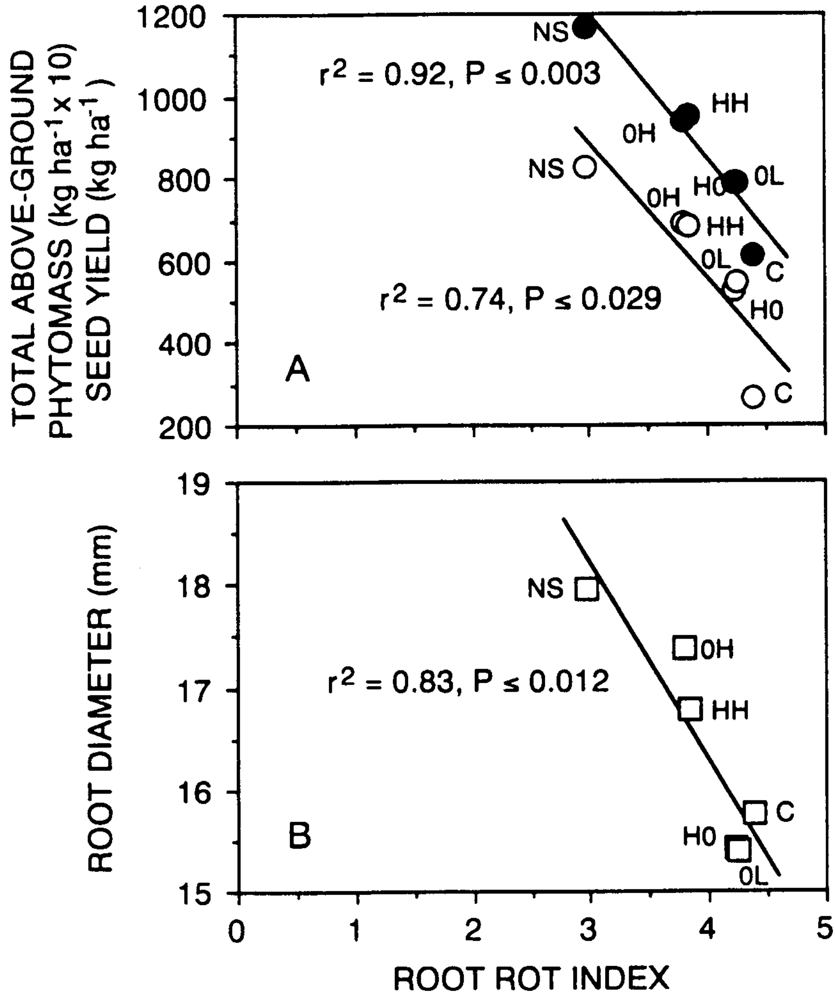


Fig. 4.5. (A) Seed yield (SY, ○), total above-ground phytomass (TAGP, ●), and (B) root diameter (□) as function of root rot index (RRI) for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1991. RRI is based on a 0 to 5 scale, where 0 = no damage and 5 = > 80% damage. Treatments are indicated beside every data point.

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CHAPTER 5

CROP WATER REQUIREMENTS AND IRRIGATION TIMING FOR WHITE CLOVER SEED PRODUCTION

ABSTRACT

White clover (*Trifolium repens* L.) seed production can be increased by constraining soil-water content during flowering. This study was conducted in 1990 and 1991 to determine white clover seed crop water requirements and supplemental irrigation timing. Six irrigation treatments assessed the influence of within- and between-season crop water requirement changes on calculated crop water stress index (CWSI) and fraction of available soil water used (FAWU) at Corvallis, OR on a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll). Under non-stressed conditions, soil water content was replenished twice weekly to field capacity with the water amount used since the last application. Four treatments had a single water replacement to 100% field capacity when 25, 46, 68 and 84% of the available water was used in 1990 and 30, 57, 64 and 79% in 1991. A non-irrigated control was also maintained. Two distinct non-stressed baselines for canopy-air temperature differences versus vapor pressure deficit were identified that were related to increasing internal hydraulic resistance during crop ageing. Maximum seed yield water use efficiency was obtained in 1990 when water application was delayed until 68% of the available soil-water was depleted. In 1991, none of the single water application treatments affected seed yield in relation to the control due to the general excessive stolon production at the beginning of the second seed production year. CWSI values across all treatments were negatively related to plant water potentials ($r^2 = 0.74$). Unlike FAWU, CWSI integrated the total plant environment and detected within-season changes in vascular resistance and leaf senescence. CWSI is a useful indicator of plant water status and can be used to schedule irrigations in white clover seed production under typical climatic conditions of western Oregon.

INTRODUCTION

The indeterminate flowering nature of white clover (*Trifolium repens* L.) grown for seed is greatly influenced by available soil-water during seed production. Seed yield is reduced by environmental conditions which favor excessive vegetative growth. Thus, crop water requirements for maximum seed yield are less than that needed for maximum vegetative growth (Clifford, 1986b). Constraining soil-water during flowering reduces vegetative growth and increases seed yield (Zaleski ,1966; Clifford, 1979, 1986b; Daynach-Deschamps and Wery, 1987; Bullita et al.,1988). Clifford (1986b) replaced available soil water to 50% of soil capacity each time "near wilting" was reached from peak flowering onwards and maintained mean plant available soil water at about 25%. Compared with non-irrigated treatments, irrigation increased seed yield by 53%. In most seed-producing areas of California, Ladino clover seed production requires from 900 to 1500 mm of irrigation water annually (Marble et al., 1970). Commonly, irrigation water is applied to replenish soil-water to root depth when the crop reaches near wilting. No information is available regarding white clover seed water use in Oregon.

Soil-based methods have been the most-used criteria for irrigation timing (Stegman, 1983). However, since many soil-plant factors affect the plant functional root system development, it is sometimes difficult to relate soil water content to crop yields. Consequently, scheduling irrigation on the basis of measured plant water stress appears to be a superior method since plant responses to both aerial and soil environments are measured (Jackson, 1981). This experiment was done to determine crop-water requirements for white clover grown for seed using plant- and soil-based methods to control irrigation timing.

Abbreviations: ASWU, average seasonal water use; CWSI, crop water stress index; DOY, day of year; ET_c, estimated crop evapotranspiration; ET_r, grass reference evapotranspiration; FAWU, fraction of available soil water used; Ψ_p , plant water potential; T_a, air temperature; T_c, canopy temperature; VPD, air vapor pressure deficit.

MATERIALS AND METHODS

The experiment was conducted as a randomized complete block design with four replications and six treatments in 1990 and 1991 at the Hyslop Research Farm near Corvallis, OR. The soil was a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll).

Each experimental unit (EU) was 4.5 m wide by 10 m long (45 m^2) and isolated by a furrow-dike system to prevent lateral surface water movement when application rates exceeded soil infiltration rate. One-meter wide alleyways at the ends of EUs were also diked. The experimental area was fumigated with methyl bromide (360 kg ha^{-1}) prior to seed bed preparation to uniformly control weeds. Ladino-type white clover 'Osceola' (Baltensperger et al., 1984) was sown 14 September 1989 in a level seedbed in single rows 0.3 m apart at a rate of 1.5 kg ha^{-1}. One 25-mm overhead sprinkler irrigation was applied after seeding to establish the crop. Seed was harvested during August and September both years.

Crop culture followed common commercial practices for western Oregon. All EUs were harvested for hay on day of year (DOY) 162 and 160 in 1990 and 1991, respectively. Haying at early-flowering synchronizes blooming with warm weather when insect pollinators are fully active and also helps control some weeds species and several insect pests. Aphids (*Nearctaphis bakeri* (Cowen)) and *Lygus* spp. were controlled with 0,0, dimethyl S-[2-ethylsulfinyl)-ethyl] phosphorothioate applied at flower bud stage at a rate of 1 kg ha^{-1}. Annual grasses and broadleaf weeds as well as volunteer clover seedlings were controlled in winter 1991 with pronamide and Gramoxone Super at a rate of 2.2 and 0.6 kg ha^{-1}, respectively. Four honey bee (*Apis mellifera* L.) hives were placed adjacent to the experimental area at the beginning of bloom time. Honey bee activity was sufficient for adequate pollination.

All EUs had similar soil-water contents at the beginning of each cropping period because rainfall during the winter maintained soil water at or above field capacity. A surface trickle irrigation system consisting of a mesh filter, ball valve, residential water flow meter, volumetric controller, and pressure regulator, distributed water to each EU. Within each EU, water was delivered through five plastic trickle lines 0.9 m apart and placed parallel to the planted rows. Each trickle line was fitted with in-line, 4 L h^{-1} turbulent-flow emitters spaced 0.9 m apart. Water was distributed to all four replicates of each treatment at the same time.

Five supplemental water application treatments were applied during the period from haying to seed harvest. The treatments were: NS) non-stressed, the soil was brought to field capacity by twice-weekly replacement of the soil water used since the previous application until 3 weeks before seed harvest; D1) single water replacement to 100% of field capacity when soil-water depletion was 25% in 1990 and 30% in 1991; D2) single water replacement to 100% of field capacity when soil-water depletion was 46% in 1990 and 57% in 1991; D3) single water replacement to 100% of field capacity when soil-water depletion was 68% in 1990 and 64% in 1991; and D4) single water replacement to 100% of field capacity when soil-water depletion was 84% in

1990 and 79% in 1991. D1, D2, D3, and D4 were grouped as single water application treatments. A non-irrigated control (C) was also maintained.

Changes in volumetric soil water content were monitored weekly by neutron attenuation (Cuenca, 1988) using 0.08-m diameter aluminum access tubes placed in the center of each EU. Readings were made at seven depths from 0.45 to 2.45 m in the high water application treatments (NS and HH), and at five depths from 0.45 to 1.55 m in the remaining treatments. The neutron attenuation probe was calibrated to the local soil conditions using gravimetric samples taken at the time of access tube installation and throughout the duration of the experiment representing a range of readings. An average soil bulk density value (1.35 g soil cm^{-3} soil $\pm$ 0.02) was obtained from five soil depths and used to convert gravimetric soil water content into volumetric values. The neutron probe counts were related to percent volumetric soil water content using the equation:

$$VWC = A\ (NMC/STD) + B$$

where VWC = volumetric soil water content (%), NMC = neutron meter count, STD = standard count for the particular neutron probe and A, B = statistical calibration coefficients (Cuenca, 1988). The resulting calibration equation was VWC = 48.4 (NMC/STD) - 21.4 (Chapter 3). Field capacity (46.03 cm^3 water cm^{-3} soil) was determined 48 h after an irrigation refilled a 2.5-m profile. Permanent wilting point (21.5 cm^3 water cm^{-3} soil) was determined in the laboratory as the water remaining in a soil sample after being subjected to 1.5 MPa of air pressure.

Seasonal estimated crop evapotranspiration (ET_c) was determined by summing applied water, precipitation, and the change in soil water content measured by neutron probe. No deep percolation was assumed. Seasonal reference evapotranspiration (ET_r) was determined from a class A evaporation pan located 200 m from the experiment site using a coefficient for different groundcover, levels of mean relative humidity, and 24-h wind (Doorenbos and Pruit, 1977). Averages daily ET_c and ET_r were calculated by dividing seasonal ET_c and ET_r by crop season length in days, respectively. Average seasonal water use (ASWU) was calculated as the ratio between seasonal ET_c and seasonal ET_r. FAWU was calculated as:

$$FAWU_i = 1 - (AW_i \ / \ TAW)$$

where $FAWU_i$ = fraction of available soil-water used of sample i, AW_i = available soil-water of sample i in mm, and TAW = total available soil-water in mm; and:

$$AW_i = (VWC_i - PWP)\ D$$

where VWC_i = volumetric soil-water content of sample i in cm^3 water cm^{-3} soil measured with a neutron probe, PWP = permanent wilting point, and D = depth of active soil profile in mm; and:

$$TAW = (FC - PWP)\ D$$

A Scheduler® Plant Stress Monitor (Carborundum Company, Solon, Ohio) measured crop canopy (T_c) and air (T_a) temperatures and air vapor pressure deficit (VPD). Four measurements per EU in all treatments were taken weekly (DOY 196 to 242 in 1990, and 184 to 251 in 1991) on sunny days between 1200 to 1400 h. Measurements were taken 1 m from the top of the canopy at a 45° oblique angle facing north, from both sides of the longer east-west axis of the EUs. CWSI was estimated using the technique of Idso et al. (1981) which is based on the relationship between T_c and T_a. The measured temperature differentials were scaled relative to the differential expected under potential evapotranspiration (non-water-stressed conditions) and the maximum differential occurring under completely suppressed evapotranspiration (fully stressed conditions). The scaled values were normalized for environmental variability with VPD. Data collected in NS treatment were used to determine the non-stressed baselines for the T_c-T_a versus VPD relationship.

The influence of inflorescence density on canopy temperatures and calculated CWSI values was evaluated in 1991. Measurements were made on DOY 200, 207, and 214 on 1.0 m^2 sections of control treatment plots with 0, 25, 50, 75 and 100% of the inflorescences sequentially removed. Measurements were taken using the same methodology described above and the target kept inside the floral density sample area.

Plant water potential (Ψ_p) measurements were made both years with a pressure chamber (Scholander et al., 1965). Three random leaves (first fully developed leaf from the stolon tip) were sampled weekly from every treatment plot between 1200 to 1500 h. In the second year, sampled leaves were wrapped in plastic clingfilm prior to excision and during measurement to minimize water loss error after excision (Leach et al., 1982).

Green leaf coverage was monitored weekly from peak flowering until harvest. Thirty samples per EU were taken with a cross-wire sighting device (Ghersa and Martinez-Ghersa, 1991) to monitor the presence or absence of green leaves in each treatment.

A 1-m wide by 8-m long (8 m^2) sample from the center of each EU was harvested with a gas-powered mower in early-morning when 80% of the florets were dry and able to shatter. Treatments NS and D4 were harvested while still flowering before fall rains began. The plant material was gathered by hand, put in burlap bags, and dried at 32 °C for 3 d. Above-ground phytomass was weighed and the seeds threshed, cleaned, and weighed. Total above-ground phytomass and seed yield water use efficiencies were calculated as the ratio of the component to seasonal ET_c.

All variables were tested by analysis of variance and simple linear regression analysis was used to relate T_a-T_c differential and VPD, Ψ_p and CWSI, and green canopy coverage and CWSI. Standard error of the mean was calculated for VMC, FAWU and CWSI measurements. Student's t pairwise comparisons were used to contrast water use efficiency means between years 1990 and 1991. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Crop water requirements. The top 1.25 m from the soil surface were considered to be the active profile based on the soil water content changes (Fig. 5.1). All water balance calculations assumed that no deep percolation occurred from the time of haying until seed harvest. The soil water content at irrigation treatment initiation was higher because of different levels of precipitation during March and April in 1991 than in 1990 (336 mm and 153 mm, respectively). In 1990, 15% of the available soil-water had been depleted at haying, while at the same time in 1991 the soil was still at field capacity. Single water application treatments were applied later in 1991 than 1990 (Fig. 5.2). As a result, crop seasons were longer for all treatments in 1991 than in 1990 (Table 5.1).

Stored soil-water depletion contributed more to the seasonal ET_c in 1991 than in 1990 due to higher soil water contents at haying. More of the total water budget was met by irrigation water in 1990 than in 1991 to maintain non water-stressed (NS) conditions (Table 5.1). Since seasonal ET_c varies with the length of the cropping season, average daily ET_c is a better comparative estimator of the water use by the different treatments. Treatment NS used more water than the rest of the treatments both years (Table 5.1). In 1990, average daily ET_c decreased with increasing irrigation depletion levels (D1 > D2 > D3 > D4). Treatments D1, D2, D3 and C had the same daily water consumption in 1991. Water applied in D4 extended the crop growing season length but was only partially used by the plants before harvest in 1990 and 1991 (Fig. 5.2). Consequently, treatment D4 average daily ET_c was lower than that of treatment C both years (Table 5.1). Average daily ET_r was lower in 1991 than 1990 for all treatments, indicating lower atmospheric demands the second year. Average daily ET_c was reduced in NS, D1, and D2 in 1991 compared to 1990 and consequently these treatments had lower ASWU values (Table 5.1). This was not apparent in the rest of the treatments. A general crop coefficient mean value of 1.05 has been cited for clover pasture crops grown in dry climates with light to moderate wind (Doorenbos and Pruit, 1977). Except for NS in 1990, all other treatments in this experiment had lower ASWU values than 1.05. For treatments D1, D2 and D3, ASWU values were similar both years with an average of 0.82 (Table 5.1). ASWU increased for D4 and C and decreased for NS in 1991 compared to 1990. Available soil-water affected plant height and crop season length (Chapter 6).Total above-ground phytomass production was not related to seasonal ET_c but was the result of the interaction between plant height and crop season length. Seasonal ET_c had no relationship with seed yield indicating the importance of water application time rather than amount in white clover seed production under western Oregon climatic conditions.

A single water application shortly before harvest (D4) maximized total above-ground phytomass water use efficiency both years (Table 5.2). Optimum seed yield (Chapter 6) and maximum seed yield water use efficiency were obtained in treatment D3 in 1990. In 1991, seed yield was unaffected by single irrigation treatments compared to the control due to the excessive stolon production during the second seed production year (Chapter 6). As a consequence, seed yield water use efficiencies were generally lower in 1991 than in 1990 and the non-irrigated control had the highest seed yield water use efficiency of all treatments the second year (Table 5.2).

Plant- and soil-based methods as irrigation timing techniques. Two non-stressed baselines which coincided with two stages of plant development were used to determine CWSI values for both years (Fig. 5.3). The in-season change in base line coincided with 50% maturity of inflorescences. Negligible changes in available soil water in the active profile during this period did not account for the increase in T_c - T_a differential with time (Fig. 5.2). Canopy temperature measurements for different inflorescence densities were used to determine whether the presence of reproductive structures affected the T_c - T_a differential as suggested by results for alfalfa seed production (Hutmacher et al., 1991). Canopy temperature measurements taken before and immediately after the removal of immature and mature flowerheads were not affected by inflorescence density (data not shown). This suggests that changes in internal hydraulic resistance during crop ageing can modify the baseline. To make CWSI measurements comparative to one another during the entire season, all values were scaled to the upper line which resulted from measured values in treatment C under severely water stressed conditions (Fig. 5.3). This empirical upper line was higher than the theoretical one calculated by the method of Idso et al. (1981).

Although the pressure chamber method is cumbersome and measurement variability great, it has generally been accepted as a fundamental measure of plant water status (Jackson, 1981; Turner, 1981). In agreement with the results cited in alfalfa seed studies (Hutmacher et al., 1991), Ψ_p values were linearly related with CWSI measurements, reflecting the usefulness of this index for quantifying plant water stress (Fig. 5.4).

Irrigation amounts were calculated to fill the entire active profile (FAWU = 0). This was not apparent for all water applications since soil water status was determined only once a week (Fig. 5.2). FAWU generally increased with time except for temporary decreases following irrigation. CWSI followed the same general pattern as FAWU but was generally lower than FAWU the first half of the flowering period and higher the second half (Fig.5.2). CWSI values generally increased faster than FAWU values as the season progressed.

Green canopy coverage was related to CWSI values ($r^2 = 0.81$; $P \leq 0.0001$). As CWSI increased, green leaves began to senesce and the transpiration rate and evaporative cooling may have decreased so the canopy temperature was higher than expected from the FAWU values. The FAWU-CWSI relationship change due to plant senescence agrees with findings for wheat (Jackson, 1981). Low CWSI values in NS were related to a constant green canopy coverage, so leaf senescence was not a causal factor for baseline changes as it is for red clover (Chapter 3).

CWSI values at the time of irrigation in single water application treatments were lower than FAWU values for early replacement dates and similar or higher for late dates (D1, D2 and D3, D4, respectively, Table 5.3). A single water application when 68% of the available water was used (CWSI = 0.46) favored reproductive expression and increased seed yield 70% in relation to the non-irrigated control (Chapter 6). CWSI not only reflected soil-water contents accurately but also revealed intrinsic plant conditions such as in-season variations in internal hydraulic resistance and leaf senescence which were not detectable with soil-based irrigation sheduling.

CONCLUSIONS

CWSI is a useful indicator of plant stress and can be used to schedule white clover seed irrigations under typical climatic conditions of western Oregon. Soil-water availability as a scheduling technique did not detect within-season plant-water requirement changes. CWSI values expressed plant conditions such as varying vascular resistance and leaf senescence during the season. CWSI irrigation scheduling was a rapid technique that integrated canopy temperatures over the entire field plot rather than rely on pressure chamber time-intensive and limited sampling. Under the conditions of this experiment, when the crop responded to supplemental water applications (1990), a single irrigation (treatment D3) filling the active soil profile at CWSI = 0.46 substantially increased seed yield compared to the non-irrigated control. For D3, the highest water-use efficiency treatment, seed crop water requirements were 310 mm. In the absence of effective stolon density control (1991), white clover seed production did not respond to supplemental irrigation.

Table 5.1. Crop season length, change in soil water content, precipitation, applied water, seasonal and average daily estimated crop evapotranspiration (ET_c), pan evaporation, seasonal and average daily reference evapotranspiration (ET_r), and average seasonal water use (ASWU) during reproductive post-haying growth for six white clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991.

Treat-ment	Crop season length	Change in soil water†	Precipi-tation	Applied water	Sea-sonal ET_c‡	Average daily ET_c§	Pan evapor-ation¶	Sea-sonal ET_r#	Average daily ET_r§	Average seasonal water use††
	d	mm								
Year 1990										
NS	81	3	56	448	507	6.26	499	412	5.09	1.23
D1	63	169	12	133	314	4.99	443	355	5.63	0.89
D2	63	181	12	101	294	4.67	443	355	5.63	0.83
D3	81	121	56	133	310	3.83	499	412	5.09	0.75
D4	81	9	56	152	217	2.68	499	412	5.09	0.53
C	57	183	12	0	196	3.44	377	306	5.37	0.64
Year 1991										
NS	99	103‡‡	69	270	442	4.46	561	475	4.80	0.93
D1	79	184	46	85	315	3.99	406	389	4.92	0.81
D2	99	190	69	127	386	3.90	561	475	4.80	0.81
D3	99	172	69	147	388	3.92	561	475	4.80	0.82
D4	99	97	69	179	346	3.49	561	475	4.80	0.73
C	71	225	46	0	271	3.82	406	345	4.85	0.79

†Data shown are soil water net change in total active profile (1.25 m deep).
‡Seasonal ET_c = water applied + precipitation + change in soil water content, during the crop season.
§Estimated as the ratio of the seasonal value with crop season length (d).
¶Data from class A evaporation pan.
#Seasonal reference evapotranspiration (ET_r) = pan coefficient x pan evaporation.
††Average seasonal water used (ASWU) = seasonal ET_c / seasonal ET_r.
‡‡Most of the change occurred between last irrigation and harvest.

Table 5.2. Total above-ground phytomass and seed yield water use efficiencies for six white clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1991 and 1991.

Treat-ment	Total above-ground phytomass water use efficiency†			Seed yield water use efficiency†		
	1990	1991	Season contrast‡	1990	1991	Season contrast
	kg ha^{-1} mm^{-1} ET_c			kg ha^{-1} mm^{-1} ET_c		
NS	15.3c§	17.1c	ns	0.7c	0.6c	ns
D1	14.8c	15.4d	ns	1.3b	1.0ab	**
D2	14.8c	17.6bc	*	1.6ab	0.8b	***
D3	22.3b	18.8b	*	1.8a	0.8b	***
D4	27.7a	20.4a	*	1.5ab	0.9b	**
C	15.2c	16.9c	ns	1.6a	1.1a	**

ns,*,**,*** Not significant, significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

†Water use efficiency is expressed as the ratio of the component with estimated seasonal crop evapotranspiration (ET_c).

‡Probability that the water use efficiency means of the two years are different according to Student's t pairwise comparison.

§Within columns, means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 5.3. Fraction of available water used (FAWU) and crop water stress index (CWSI) at irrigation time for white clover seed single water application treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991.

	Year 1990			Year 1991		
Treatment	Day of year	FAWU†	CWSI‡	Day of year	FAWU†	CWSI‡
		- fraction of maximum -			- fraction of maximum -	
D1	170	0.25	-§	189	0.30	0.20
D2	192	0.46	-	210	0.57	0.48
D3	204	0.68	0.46	220	0.64	0.64
D4	218	0.84	0.95	231	0.79	0.91

†FAWU average standard error = 0.012.

‡CWSI average standard error = 0.028.

§Data not taken.

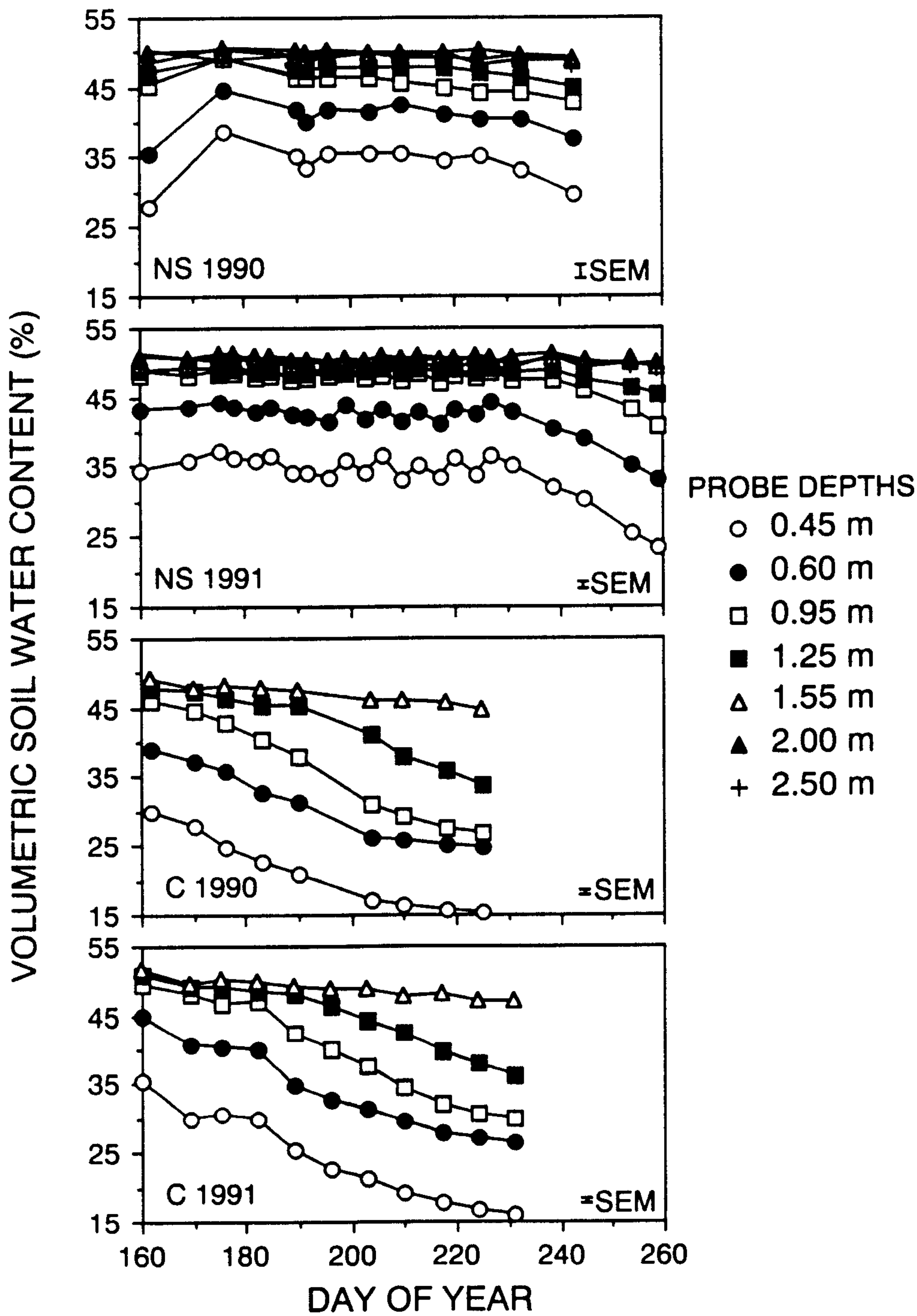

Fig. 5.1. Variation of volumetric soil water content with day of year at specific soil depths in non-stressed (NS) and non-irrigated (C) treatments for white clover seed at Hyslop Farm, Corvallis, OR in 1990 and 1991. Vertical bars indicate average standard error of the mean (SEM).

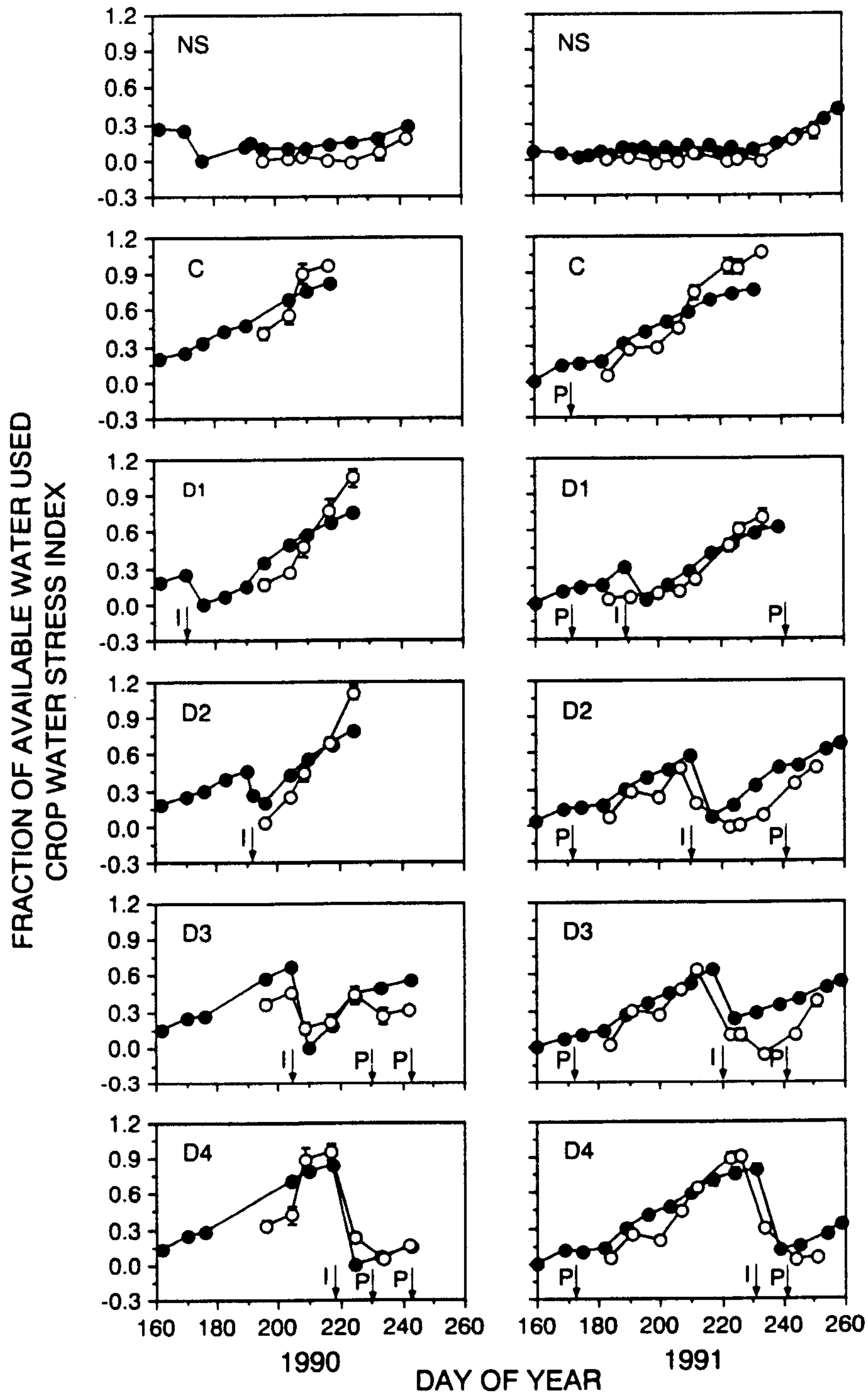

Fig. 5.2. Crop water stress index (o) and fraction of available soil-water used (●) as functions of day of year for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991. Vertical bars indicate standard error of the mean. Arrows labelled I and P indicate irrigation application and precipitation (<10 mm) dates, respectively (not shown for treatment NS).

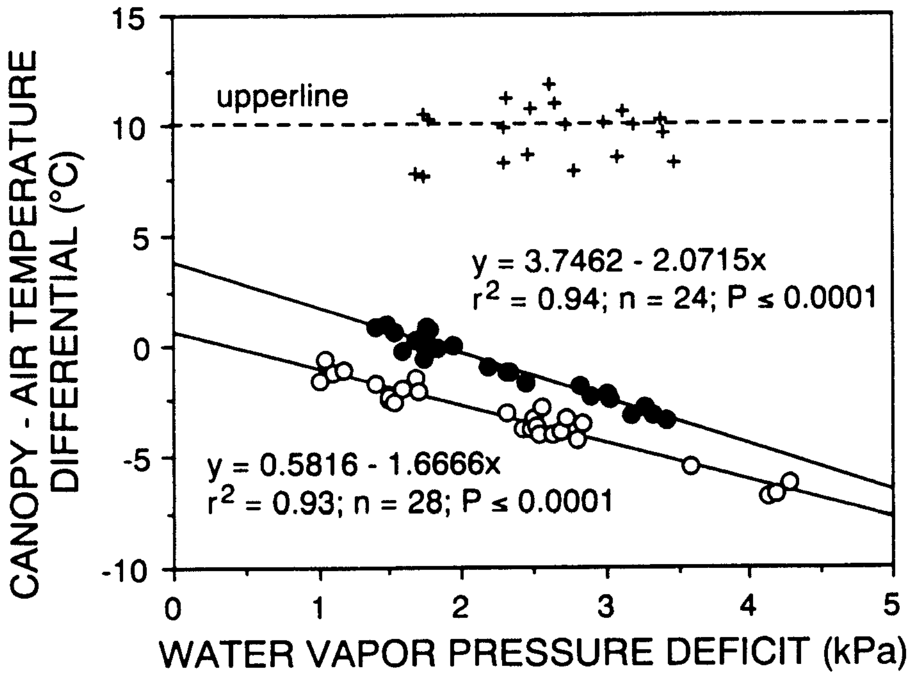

Fig. 5.3. Non-stressed baselines for canopy temperature minus air temperature versus water vapor pressure deficit determined using data from the non-stressed treatment before (o) and after (●) 50% of the white clover inflorescences were mature at Hyslop Farm, Corvallis, OR in 1990 and 1991. The upperline is based on data from non-irrigated control (+) at the end of the cropping seasons.

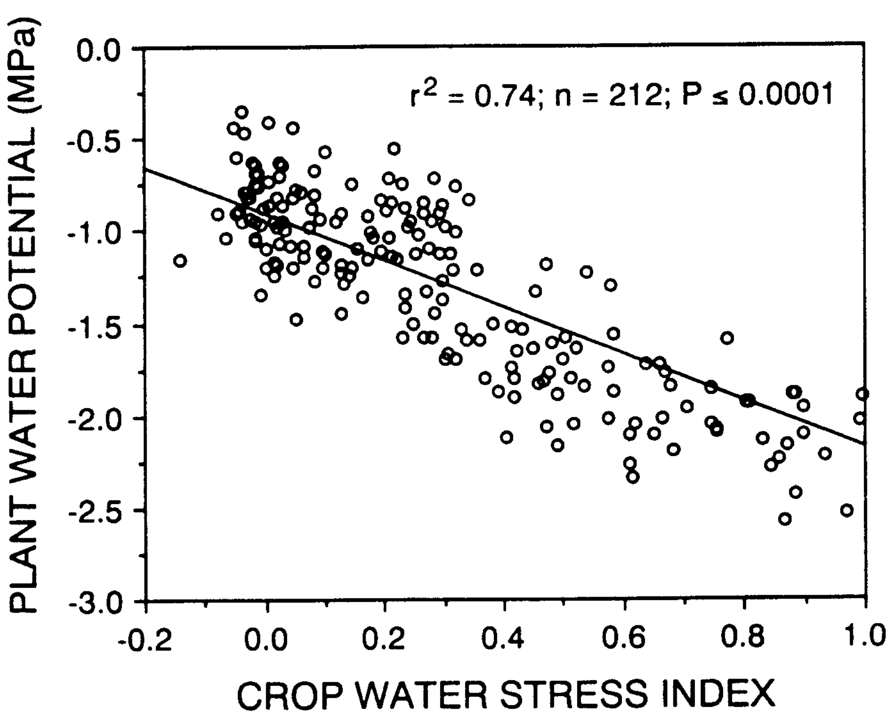

Fig. 5.4. Regression of plant water potential on crop water stress index from six white clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1991. Measurements were from days of year 190 to 255.

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CHAPTER 6

SOIL AND PLANT WATER STATUS EFFECTS ON WHITE CLOVER SEED YIELD AND YIELD COMPONENTS

ABSTRACT

White clover (*Trifolium repens* L.) seed yield is reduced by environmental conditions which favor excessive vegetative growth and consequently reduce inflorescence density. White clover seed yield can be highly variable and low in western Oregon. The objective of this study was to quantify the effects of soil and plant water status on white clover inflorescence production, seed yield, and seed yield components. Five supplemental irrigation treatments were applied in 1990 and 1991 to first and second year white clover grown on a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll) near Corvallis, OR. In the non-stressed treatment, the soil water content was brought to field capacity by twice weekly replacement of water used since the last application. Four treatments had a single water replacement to 100% field capacity when 25, 46, 68 and 84% of the available water was used in 1990 and 30, 57, 64 and 79% in 1991. A non-irrigated control was also maintained. Seed yield (SY) was optimum in 1990 when water application was delayed until 68% of the available soil-water was used by the crop which maintained an even flush of flowers and restricted vegetative growth. In 1991, all irrigation treatments yielded the same or less than the non-watered control. This was due to the excessive vegetative growth from stolons that had grown between the planted rows the previous and present crop year. In both years excessive amounts of irrigation water favored profuse vegetative growth and reduced SY. Irrigating too late in the season increased total above-ground phytomass (TAGP) but decreased seed yield. Inflorescence density was increased by constraining soil-water in 1990 and was the yield component that most affected SY both years. In 1990, proper depletion water management increased SY 70% and potential seed yield (SY_{pot}) utilization 7% in relation to

the non-irrigated control. Under conditions which result in excessive stolon development prior to the second reproductive season, aggressive vegetation management is needed to increase inflorescence density and seed yield.

INTRODUCTION

White clover grows indeterminately from stolon tips which form either secondary stolons or inflorescences (Thomas, 1961). Basic vegetative growth functions affect reproductive expression (Clifford, 1980, 1985, 1986a). Excessive foliage growth from summer irrigation can reduce reproductive expression (Clifford 1985). Constraining soil water during the flowering period reduces vegetative growth so more inflorescences are formed giving higher seed yields (Zaleski ,1966; Clifford, 1986a; Daynach-Deschamps and Wery, 1987; Bullita et al.,1988). Clifford (1979, 1986b) found during first-year canopy formation that reduced soil-water availability during reproduction limited plant nutrient uptake, reduced leaf size, and increased inflorescence density. Decreased soil water availability also reduced the number of florets per inflorescence but this yield component reduction was completely offset by increased inflorescence density (Clifford, 1979, 1986b). The proportion of ovules that set seeds in white clover is normally low (Thomas, 1987). The causes of low seed set are not known but a combination of high temperatures and decreased soil water availability can reduce the number of ovules per carpel (Thomas, 1981b). With irrigation, ovule abortion is reduced and seed weight increased compared to the non- irrigated conditions (Clifford, 1986b).

The purpose of this research was to quantify the effects of plant and soil water status on inflorescence production, seed yield, and yield components for white clover grown in western Oregon.

Abbreviations: SY_{act}, actual seed yield; CWSI, crop water stress index; ET_c, estimated crop evapotranspiration; FAWU, fraction of available soil-water used;

HI, harvest index; SY_{pot}, potential seed yield; SBF, seed bearing flowers; SY, harvested seed yield; TAGP, total above-ground phytomass.

MATERIALS AND METHODS

The experiment was conducted as a randomized complete block design with four replications and six treatments for two years at the Hyslop Research Farm near Corvallis, OR. The soil was a Woodburn silt loam (fine-silty, mixed, mesic Aquultic Argixeroll).

Each experimental unit (EU) was 4.5 m wide by 10 m long (45 m^2) and isolated by a furrow-dike system to prevent lateral surface water movement when application rates exceeded soil infiltration rate. The experimental area was fumigated with methyl bromide (360 kg ha^{-1}) prior to seed bed preparation to uniformly control weeds. Ladino-type white clover 'Osceola' (Baltensperger et al., 1984) was sown 14 September 1989 in a level seedbed in single rows, 0.3 m apart, at a rate of 1.5 kg ha^{-1}. One 25-mm irrigation was applied by overhead sprinklers after seeding to establish the crop. The EUs were harvested for seed during August and September in 1990 and 1991.

Crop culture followed common commercial practices. All EUs were harvested for hay in the spring (DOY 162 and 160 in 1990 and 1991, respectively). Haying at early-flowering better synchronizes bloom with warm weather when insect pollinators are fully active and helps control some weeds species and several insect pests. Aphids (*Nearctaphis bakeri* (Cowen)) and *Lygus* spp. were controlled with 0,0, dimethyl S-[2-ethylsulfinyl)-ethyl] phosphorothioate applied at bud stage of development at a rate of 1 kg ha^{-1}. Annual grasses and broadleaf weeds were controlled in winter 1991 with pronamide and Gramoxone Super at a rate of 2.2 and 0.6 kg ha^{-1}, respectively.

Four honey bee (*Apis mellifera* L.) hives were placed adjacent to the experimental area at the beginning of bloom time. Honey bee activity was sufficient for adequate pollination.

All EUs had similar soil-water contents at the beginning of each cropping period because rainfall during the winter maintained soil water at or above field capacity. A surface trickle irrigation system consisting of a mesh filter, ball valve, residential water flow meter, volumetric controller, and pressure regulator, distributed water to each EU. Within each EU, water was delivered through five plastic trickle lines 0.9 m apart and placed parallel to the planted rows. The trickle lines were fitted with in-line turbulent-flow emitters spaced 0.9 m apart that delivered 4 L of water h^{-1}. Water was distributed to all four replicates of each treatment at the same time.

Five supplemental water application treatments were applied during the cropping period from haying to seed harvest. The treatments were: NS) non-stressed, the soil was brought to field capacity by twice-weekly replacement of the soil water used since the last application until 3 weeks before seed harvest; D1) single water replacement to 100% of field capacity when the fraction of available soil-water used (FAWU) was 0.25 in 1990 and 0.30 in 1991; D2) single water replacement to 100% of field capacity when FAWU was 0.46 in 1990 and 0.57 in 1991; D3) single water replacement to 100% of field capacity when FAWU was 0.68 in 1990 and 0.64 in 1991; and D4) single water replacement to 100% of field capacity when FAWU was 0.84 in 1990 and 0.79 in 1991. D1, D2, D3, and D4 were considered to be single depletion water application treatments. A non-irrigated control treatment (C) was also maintained.

Seasonal estimated crop evapotranspiration (ET_c) was determined by the summation of applied water, precipitation, and the change in soil-water content determined by neutron probe measurements (Chapter 5). FAWU values were calculated as described in Chapter 5. Plant-water status was monitored from the beginning of flowering until harvest using infrared thermometry (Idso et al., 1981). Crop water stress index (CWSI) values were determined as described in Chapter 5. Average seasonal CWSI and FAWU were calculated as the means of weekly measurements taken during the reproductive period.

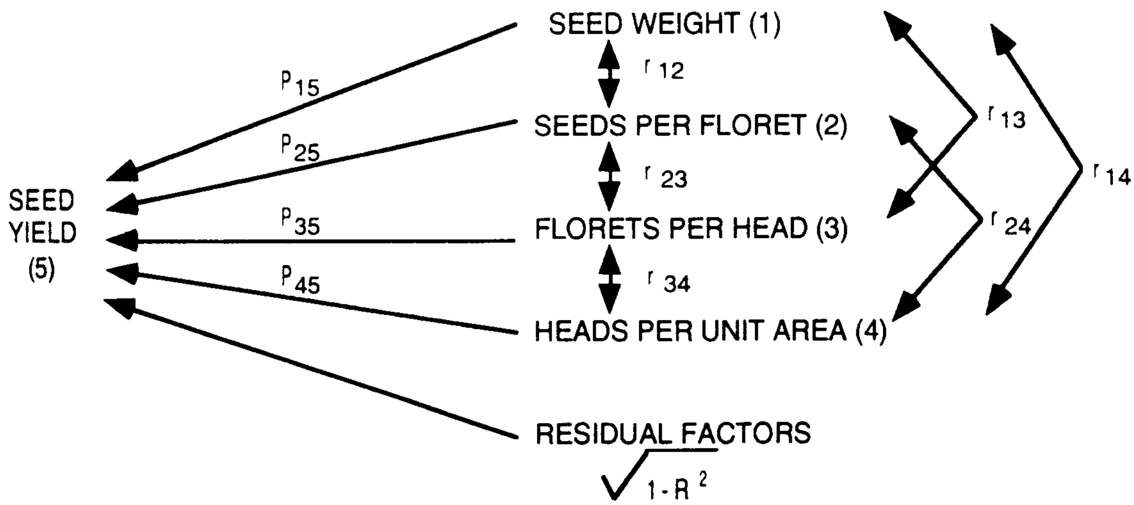
In both years the number of inflorescences in three 0.1 m^2 random samples per EU were counted weekly. Plant height was also measured. Floral phenological stages were rated as: 1) floral bud: youngest floral meristem (visible bud) to fully expanded head with less than 50% opened florets; 2) flowering head: majority of florets opened to less than 50 % desiccated florets; and 3) seed bearing flowers (SBF): majority of florets desiccated to dry seed. Peak flowering was determined as the time when the crop displays a maximum number of inflorescences in anthesis. Average plant height was calculated as the mean of weekly measurements.

A 1-m wide by 8-m long (8 m^2) sample from the center of each EU was harvested with a gas-powered mower in early-morning when 80% of the florets were dry and able to shatter. Treatments NS and D4 were harvested while still flowering before fall rains began. The plants were cut and material gathered by hand and put in burlap bags in early morning to minimize shattering and dried at 32°C for 3 days. TAGP was weighed and the seeds threshed, cleaned, and weighed to obtain SY. Seeds were stored at 10° C and 35% RH. Harvest index (HI) was calculated dividing SY by TAGP.

At harvest time, three 0.1 m^2 random samples per EU were taken to determine the number of florets per inflorescence (florets from 10 random flower heads per sample) and the number of seeds per pod (50 random florets per sample). Forage legume SY_{pot} is defined as the number of ovules (or potential seed sites) per unit ground area at anthesis (Lorenzetti, 1981) and was calculated as the product of the number of inflorescences per unit area, florets per inflorescence, unit seed weight, and a theoretical six seed sites per pod. Actual seed yield (SY_{act}) was calculated using the actual seed per pod values.

Seed moisture content was determined from two random 5-g samples per EU dried at 130 °C for 1 h (Grabe, 1989). Mean seed weight was determined from three samples of 200 seeds. Germination percentage was estimated from 3 replicates of 50 seeds on blue blotter in plastic sandwich box placed in chambers at 19 °C for 7 d (Association of Official Seed Analysts, 1978). Seed vigor was estimated with 20 seeds per EU placed on a line drawn 35 mm from the upper end of a moist blotter inside a polystyrene box. The boxes were held at 70° angle from the horizontal in a chamber at constant 19 °C. Number of germinated seeds were counted daily until day 7 and mean germination time (mean germination time = $\Sigma X_i T_i / \Sigma X_i$, where X_i = number of newly germinated seeds at time T_i) calculated. On the seventh day, radicle and plumule lengths of all seedlings with visible radicles were measured with a 10 x 10 cm, 2 mm^2 grid, plastic transparency (Oliva et al., 1987). Before testing, all seeds were scarified on sandpaper under a 50 PSI air flow for 30 s.

Seed yield components were subjected to path-coefficient analysis across all treatments to partition the correlation coefficients into direct and indirect components (Dewey and Lu, 1959). This information was used to determine the influence of plant water stress on the relative importance of each seed yield component. The five variables included in the path-coefficient analysis and the direction of their causal relationship are represented by:



Double-arrowed lines indicate mutual associations which are measured by correlation coefficients (r_{ij}), and single-arrowed lines represent direct effects as measured by path coefficients (P_{ij}).

All variables were tested by analysis of variance. Simple linear regression analysis was used to relate TAGP with average seasonal CWSI and the product of average plant height and crop season length, and plant height with average seasonal FAWU. Standard errors of the mean were calculated for number of flowering heads, SBF, and CWSI. Student's t pairwise comparisons were used to contrast average SY, TAGP, and HI between years 1990 and 1991. All differences reported are significant at $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Inflorescence production and development. All treatments began to flower a week after haying and reached peak flowering at DOYs 186 and 182 in 1990 and 1991, respectively (Fig. 6.1). The indeterminate flowering nature of white clover plants was greatly influenced by the amount of soil-water available to the crop during flowering and seed production. In 1990, 15% of the available soil water had been depleted at haying, while at that time in 1991 the soil was still at field capacity (Chapter 5). Irrigation treatments were delayed in 1991 until soil-water depletion levels were similar to those in 1990 (Fig. 6.1). As a consequence of higher soil-water availability in 1991, the duration of the season-long flower production increased for all treatments compared to 1990. In both years, the duration of the reproductive period increased with decreasing crop water stress levels. Treatments NS and D3 maintained an even flush of flowers throughout the growing season because of low water stress during reproduction as measured by CWSI (Fig. 6.1). No irrigation (C) or water application prior to that of treatment D3 (D1, D2) generally resulted in shorter flowering periods. Delaying irrigation further into the season (D4) coincided with harvest time for unirrigated treatment C. Plants in treatment D4 reinitiated flowering but did not mature seeds before the first rains in the fall. The number of SBF decreased for all treatments in 1990 compared to 1991 (Fig.6.2). SBF generally increased with increasing duration of the flowering period in 1990 but not in 1991. In both years, the rate of SBF production was lowest for NS and similar for the rest of the treatments. However, late water application (D4) increased the number of mature inflorescences lost under new-growth canopy and did not produce new SBF at harvest time (Fig. 6.2).

Seed yield. SY was optimum in 1990 when water application was delayed until 68% of the available soil-water was used by the crop (D3, Table 6.1). Earlier water replacement (D1, D2) or no supplemental water (C), reduced SY. Treatment D4 yielded even less than the non-irrigated control because of the increased seed loss due to inflorescence rotting under the canopy. The excess of vegetative development in the non-stressed treatment (NS) competed for space with reproductive stolons and reduced SY to levels of the control. Deficit irrigation water has been shown to be needed for the first-year seed crop to build up a balanced stolon density to achieve a high ratio of reproductive to vegetative apical meristems (Clifford, 1987).

In 1991, all single water application treatments (D1, D2, D3, D4) yielded the same, and the treatment NS less than the non-watered control (Table 6.1). This was due to excessive vegetative growth from stolons that grew between the planted rows in all treatments the previous year and prior to haying in 1991. Conventional haying did not effectively re-establish the planted rows, so the balance between reproductive and vegetative development needed for optimum seed production was not maintained. Subsequently SBF production was reduced (Fig. 6.2). It has been shown that an over-dense crop canopy in late-autumn and early-spring will reduce inflorescence production, particularly in second year crops (Zaleski, 1961; Hides et. al., 1984; Marshall and James, 1988).

There was no predictable relationship between seasonal ET_c, FAWU, or CWSI and seed yield indicating the importance of timing rather than water application amount in white clover seed production (Table 6.1). Constraining soil-water during the flowering period increased inflorescence production and seed yield in accordance with the finding of others (Hagan et al., 1957; Zaleski, 1966; Adachi and Suzuki, 1968; Clifford, 1979, 1986b; Daynach-Deschamps and Wery, 1987; Bullita et al.,1988). In 1990, delaying water application until a FAWU value $\approx$ 0.65 was reached increased seed yields compared to other treatments (Fig. 6.3). This equated to a seasonal FAWU value of 0.4 (mean available soil-water = 60 %) and a seasonal CWSI of 0.3 in 1990 (Table 6.1). Clifford (1987) recommended replacing available soil-water to 50% of soil capacity each time "near wilting" is reached from the time of peak flowering and later. This system maintained mean plant available soil water at about 25% and increased seed yield by 53% compared to the non-irrigated treatments in a first-year seed crop (Clifford, 1986b). Under western Oregon climatic conditions, white clover seed plants nearly reached wilting point at the end of the growing season (Chapter 5). Full replacement of the soil-water used was necessary to maintain a constant flowering until near harvest when a single supplemental irrigation is applied (Fig. 6.1).

TAGP production decreased with increasing average seasonal CWSI in both years (Fig. 6.4). Average seasonal plant height decreased with increasing seasonal FAWU (r^2 = 0.91 and 0.88 in 1990 and 1991, respectively) and the length of the cropping season increased in treatments with low CWSI values toward the end of the season (NS, D3, D4, Fig. 6.1). TAGP was the result of the interaction between average seasonal plant height and crop season length (Fig. 6.4). In 1990, increased TAGP production increased SY (D3 vs. C, Table 6.1), but excessive irrigation (NS) favored profuse vegetative growth and reduced SY. If water application was delayed until late-season (D4) TAGP increased but SY decreased. TAGP production was the same or greater and SY was the same or less for all treatments in 1991 than in 1990 (Table 6.1). As a result, HI was lower for all treatments in 1991 than in 1990. The non-irrigated control had the maximum reproductive efficiency in both years (Table 6.1).

Components of seed yield and seed yield potential. Inflorescences per unit area and seed weight were the only seed yield components affected by irrigation treatments in 1990 (Table 6.2). Clifford (1979, 1986b) found that deficit irrigation increased first-year seed yield by increasing inflorescence density and floral fertility (seeds per floret). In the present study, inflorescence density increased in single water application treatments (D1, D2 and D3) compared to the non-irrigated (C) and non-stressed (NS) treatments (Table 6.2). Seed weight increased in all watered treatments compared to the non-irrigated control. In 1991, inflorescence density was reduced by excessive irrigation water (NS) but not affected by single water application treatments (D1, D2, D3, Table 6.2). Due to the generalized excessive vegetative growth, inflorescence density was lower in 1991 than in the previous year for all treatments. Competition between reproductive and vegetative growth may have reduced floral fertility in 1991 compared to 1990 (Table 6.2). Except for inflorescence density, all other seed yield components were not related to water management in 1991. These results were confirmed by path-coefficient analysis across all treatments (Table 6.3). In 1990, seed yield was similarly affected by the direct effects of seed weight and inflorescences per unit area. In 1991, inflorescence density was the only cause of SY variations. This substantiates the finding that the number of ripe inflorescences at harvest is the most important factor in obtaining satisfactory seed yields (Zalesky, 1970; Evans et al., 1986; Hollington et al., 1989).

Yield component responses to different irrigation treatments did not affect seed quality. Average treatment mean time to germination was 2.9 d $\pm$ 0.3 in 1990 and 3.1 d $\pm$ 0.2 in 1991. Total seedling length at day seven of germination was 32 mm $\pm$ 2 and 34 mm $\pm$ 1 in 1990 and 1991, respectively. Germination percentage for all treatments after scarification was 98.4% $\pm$ 0.7 in 1990 and 99.1 $\pm$ 0.3 in 1991.

SY_{pot} was calculated using a theoretical six seed sites per floret. Consequently, variations in SY_{pot} among treatment was due to the water management effects on inflorescence number per unit area, florets per inflorescence, and seed weight. SY_{pot} in 1990 was maximum for single water application treatments (D1, D2, D3) due to higher inflorescence density (Table 6.2). In 1991, among-treatment differences in number of inflorescences per unit area was compensated by other yield components so SY_{pot} was the same for all treatments. The general decrease in number of ripe inflorescences at harvest in 1991 compared to 1990 accounted for the lower second-year SY_{pot}. The differences between SY_{pot} and SY_{act} were due to reduced actual floral fertility. Reduced floral fertility for all treatments in 1991 compared to 1990 resulted in lower SY_{act} as percentage of SY_{pot} in 1991. Values of SY_{act} (29 to 47%) and SY (12 to 27%) as percentages of SY_{pot} were lower for all treatments than the values (50% and 35%, respectively) cited for white clover by Lorenzetti (1981). However, due to the considerable genetic variations for seed yield components among white clover cultivars (Van Bochstaele and Rijckaert, 1988), this comparison would be more useful within similar genotypes. SY as percentage of SY_{pot} was slightly higher for D3 than for the rest of the treatments in 1990, while all treatments had similar values in 1991 (Table 6.2). Water stress effects alone evidently cannot explain differences between SY_{pot} and SY. In 1990, proper water management improved SY from 318 kg ha^{-1} (C) to 543 kg ha^{-1} (D3), which represented 70% increase in SY and a 7% improvement in the utilization of the SY_{pot}.

CONCLUSIONS

Irrigation water was needed for a fall-planted first-year white clover seed crop to achieve the best balance between reproductive and vegetative growth under western Oregon climatic conditions. A single water application when about 65% of the available soil-water was used increased seed yields 70% compared to the non-irrigated control. However, some plant water stress (average seasonal CWSI $\approx$ 0.32) was essential to increase inflorescence density and reduce leaf production. The same water management schemes during the second-year seed crop allowed an even flush of flowers throughout the growing season, but did not increase seed yield compared to the control. Under conditions which result in excessive stolon development prior to the second seed production season, aggressive vegetation management would be needed in addition to optimum water management to achieve high inflorescence density and seed yield.

Table 6.1. Crop season length, average plant height, seed yield (SY), total above-ground phytomass (TAGP), harvest index (HI), average seasonal crop water stress index (CWSI), seasonal estimated crop evapotranspiration (ET_c), and average seasonal fraction of available soil-water used (FAWU) for six white clover seed irrigation treatment at Hyslop Farm, Corvallis, OR in 1990 and 1991. Season contrasts for average plant height, seed yield, total above-ground phytomass and harvest index are shown.

Treatment	Crop season length	Average plant height	Seed yield	TAGP	Harvest index	Average seasonal CWSI	Seasonal ET_c	Average seasonal FAWU
	d	m	- 1000-kg ha^{-1} -				mm	
Year 1990								
NS	81	0.36a†	0.35bc	7.75a	4.5c	0.04	507	0.12
D1	63	0.27b	0.42bc	4.65d	9.0b	0.55	314	0.41
D2	63	0.27bc	0.45ab	4.36d	10.4a	0.50	294	0.47
D3	81	0.25cd	0.54a	6.90b	7.9b	0.32	310	0.41
D4	81	0.25d	0.33c	6.01c	5.5c	0.43	217	0.49
C	57	0.24d	0.32c	2.98e	10.7a	0.71	196	0.61
Year 1991								
NS	99	0.37a	0.24b	7.56a	3.2c	0.04	442	0.15
D1	79	0.33b	0.30a	4.83c	6.2a	0.33	315	0.33
D2	99	0.33b	0.31a	6.80b	4.6b	0.21	386	0.39
D3	99	0.32c	0.32a	7.30ab	4.3b	0.23	388	0.38
D4	99	0.30d	0.29ab	7.06ab	4.1b	0.37	346	0.41
C	71	0.30d	0.30a	4.56c	6.7a	0.63	271	0.51
Season contrast								
NS		ns	ns	ns	ns			
D1		**	**	ns	**			
D2		**	**	***	**			
D3		**	***	ns	**			
D4		**	ns	ns	ns			
C		***	ns	***	**			

ns, *,**,***Not significant, significant at 0.05, 0.01, 0.001 probability levels, respectively.

†Within columns, means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 6.2. Seed yield components for six white clover irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991.

Treat-ments	Inflor-escences	Florets per inflor-escence	Seeds per floret	Seed weight	Seed yield: Potential†	Seed yield: Actual‡	Seed yield: Harvested§	Seed yield as percentage of potential: Actual	Seed yield as percentage of potential: Harvested
	no. m^{-2}			mg	$g\ m^{-2}$			%	
Year 1990									
NS	675d¶	105a	2.20a	0.59a	252c	95b	35bc	37a	14bc
D1	910ab	107a	2.80a	0.57b	330ab	153a	41bc	47a	13bc
D2	958a	104a	2.60a	0.58ab	344a	147a	45ab	43a	13bc
D3	818bc	100a	2.51a	0.58ab	282abc	118ab	54a	42a	19a
D4	681d	97a	2.38a	0.56b	223c	89b	33c	40a	15b
C	763cd	110a	2.72a	0.54c	270bc	124ab	32c	45a	12c
Year 1991									
NS	314b	106a	1.76c	0.58a	115a	34c	24b	29c	21a
D1	433a	92b	2.14ab	0.59a	139a	49ab	30a	36ab	22a
D2	400ab	99ab	1.77c	0.59a	139a	41bc	31a	29c	23a
D3	408ab	89b	1.93bc	0.57a	124a	40bc	32a	32bc	27a
D4	332b	105a	2.30a	0.57a	119a	46bc	29ab	38a	24a
C	443a	107a	2.15ab	0.58a	166a	60a	30a	36ab	19a

†Potential seed yield = inflorescences per m^2 x florets per inflorescence x seed weight x 6 (potential seed sites per floret).

‡Estimated using actual number of seeds per floret.

§Seed harvested and cleaned.

¶For each year, within-column means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

Table 6.3. Path coefficient analysis across all white clover seed irrigation treatments at Hylsop Farm, Corvallis, OR in 1990 and 1991.

Pathway	1990	1991
Seed weight *vs.* seed yield		
Direct effect, P_{15}	0.52**	0.03ns
Indirect effects		
via seeds per floret, $r_{12}P_{25}$	-0.13	0.01
via florets per inflorescence, $r_{13}P_{35}$	0.01	0.00
via inflorescences per unit area, $r_{14}P_{45}$	0.02	0.13
Correlation, r_{15}	0.42*	0.17ns
Seeds per floret *vs.* seed yield		
Direct effect, P_{25}	0.28ns	0.04ns
Indirect effects		
via seed weight, $r_{12}P_{15}$	-0.25	0.01
via florets per inflorescence, $r_{23}P_{35}$	-0.01	0.00
via inflorescences per unit area, $r_{24}P_{45}$	0.14	0.02
Correlation, r_{25}	0.16ns	0.07ns
Florets per inflorescence *vs.* seed yield		
Direct effect, P_{35}	-0.04ns	0.02ns
Indirect effects		
via seed weight, $r_{13}P_{15}$	-0.16	0.00
via seeds per floret, $r_{23}P_{25}$	0.08	0.00
via inflorescences per unit area, $r_{34}P_{45}$	0.11	-0.17
Correlation, r_{35}	-0.02ns	-0.15ns
Inflorescences per unit area *vs.* seed yield		
Direct effect, P_{45}	0.49**	0.68**
Indirect effects		
via seed weight, $r_{14}P_{15}$	0.03	0.00
via seeds per floret, $r_{24}P_{25}$	0.08	0.00
via florets per inflorescence, $r_{34}P_{35}$	-0.01	0.00
Correlation, r_{45}	0.59**	0.68**

ns,*,**Not significant, significant at the 0.05 and 0.01 probability levels, respectively.

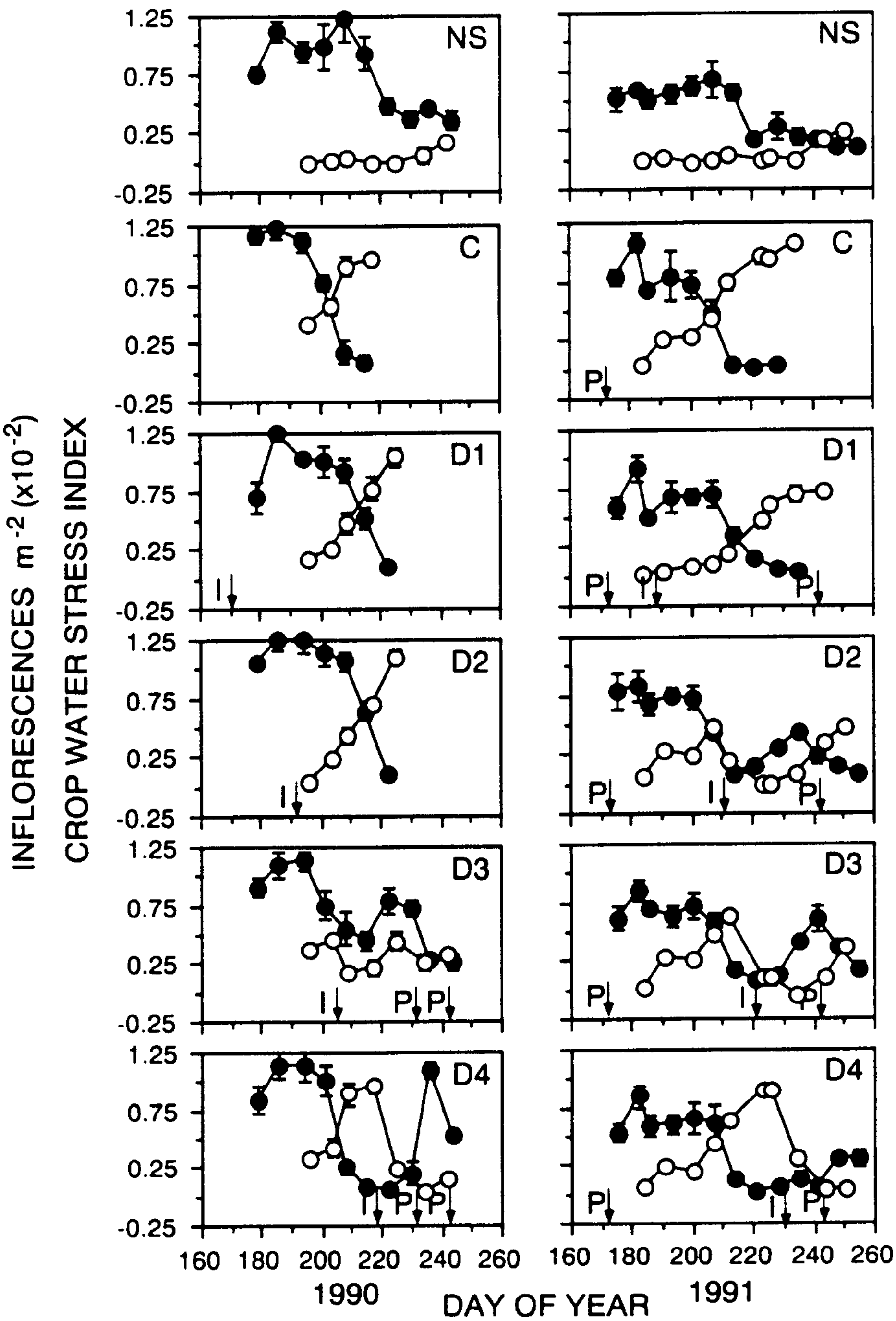


Fig. 6.1. Number of flowering heads (●) and crop water stress index (○) as function of day of year for six white clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 and 1991. Vertical bars indicate standard error of the mean. Arrows labelled I and P indicate irrigation application and precipitation (>10 mm) dates, respectively (not shown for treatment NS).

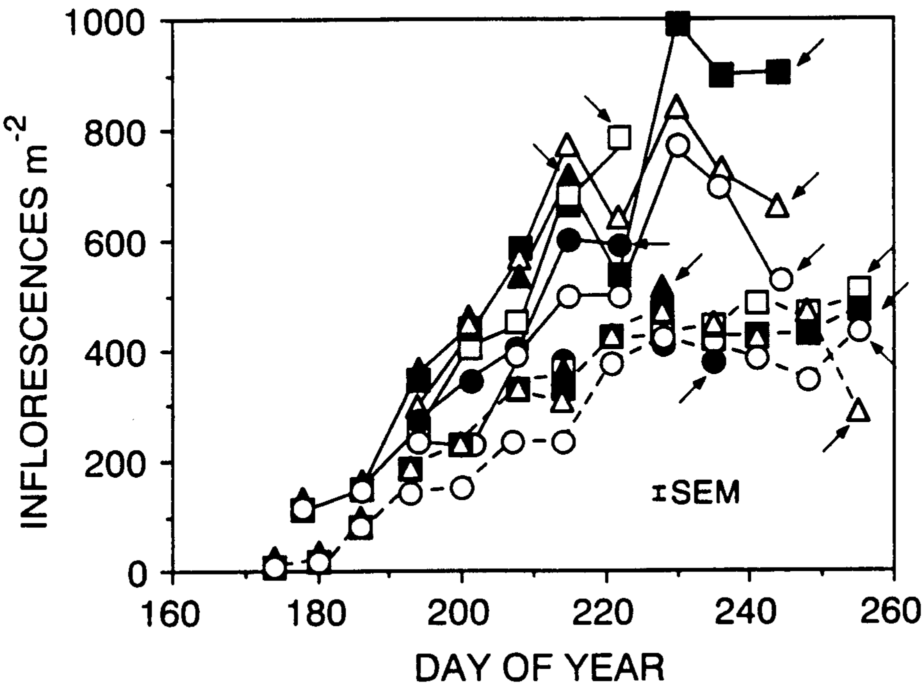


Fig. 6.2. Seed bearing flowers (SBF) as function of day of year for six white clover irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (——) and 1991 (- - -). ○ NS, ● D1, □ D2, ■ D3, Δ D4, and ▲ C. Arrows identify number of SBF at harvest for each treatment in both years. Vertical bar indicates average standard error of the mean (SEM).

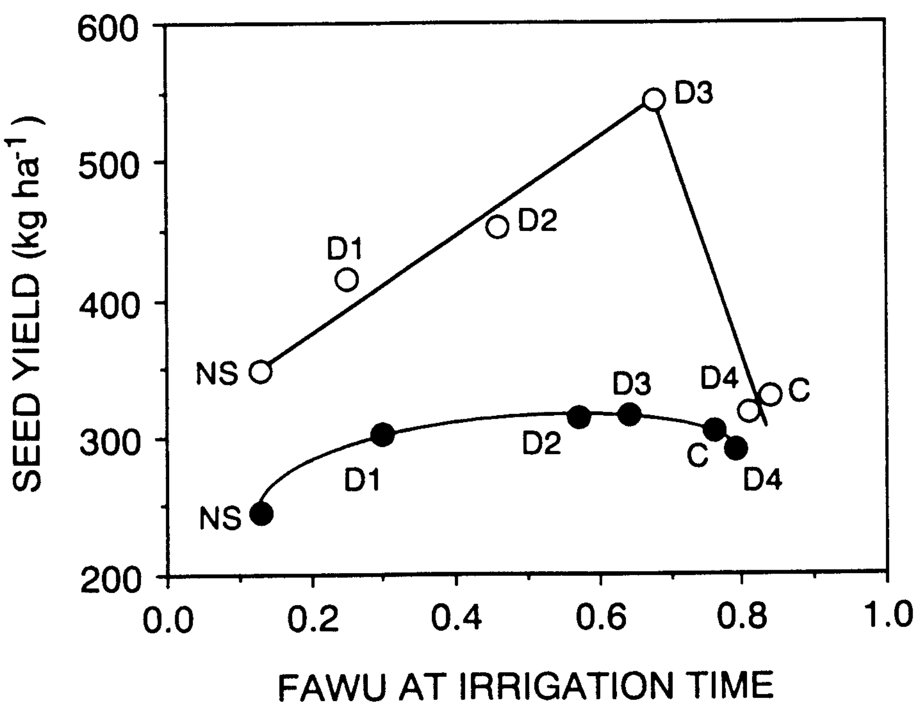


Fig. 6.3. Seed yield as function of fraction of available soil water used (FAWU) at the time of irrigation for six white clover irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (○) and 1991 (●). Hand-drawn lines show the trend of seed yield increases with delaying irrigation up to a FAWU value ~ 0.65. FAWU values for treatment NS are the all-irrigation averages, and for treatment C are the fractions used at harvest time. Treatments are indicated beside every data point.

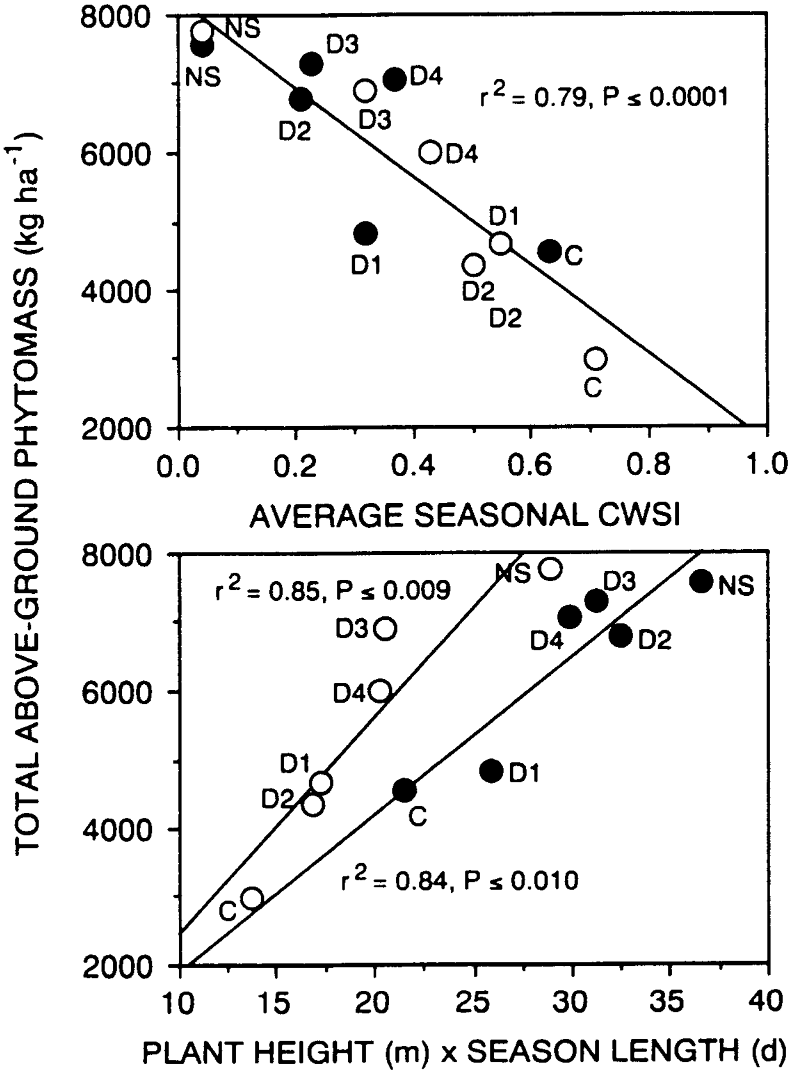


Fig. 6.4. Total above-ground phytomass (TAGP) as function of average crop water stress index (CWSI) and the product of average plant height by crop season length for six white clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (○) and 1991 (●). Treatments are indicated beside every data point.

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APPENDIX

Appendix Table 1. Monthly and total precipitation data, and its departure from normal values, for 1989-90 and 1990-91 growing seasons at Hyslop Farm, Corvallis, OR.

	Growing season 1989-90		Growing season 1990-91	
Month	Precipitation	Departure	Precipitation	Departure
	mm			
September	134	-22	21	-17
October	68	-19	116	30
November	99	-58	124	-33
December	78	-119	90	-107
January	241	50	68	-124
February	147	24	82	-42
March	56	-61	149	31
April	60	-2	88	26
May	36	-12	99	51
June	39	8	39	8
July	11	4	10	2
August	44	23	18	-2
Total	895	-186	904	-177

Appendix Table 2. Crop water stress index (CWSI) values for red clover seed control plots with 0, 25, 50, 75 and 100% of mature and flowering heads sequentially removed on peak flowering time ± 7 days at Hyslop Farm, Corvallis, OR in 1991.

Day of year	Inflor-escences at anthesis/ at pod-filling	Percent of heads removed				
		0	25	50	75	100
	no. m^{-2}	CWSI†				
200	135/21	0.15±0.01	0.16±0.02	0.17±0.01	0.17±0.01	0.16±0.01
207	270/80	0.26±0.01	0.28±0.01	0.26±0.01	0.28±0.01	0.26±0.01
214	235/331	0.47±0.01	0.47±0.01	0.45±0.01	0.46±0.01	0.45±0.01

†The non-stressed baseline used was: Tc - Ta = 1.59 - 1.54*VPD, where canopy (Tc) and air (Ta) temperatures are in °C and vapor pressure deficit (VPD) is in kPa. All measurements were made under cloud-free conditions within 2 h after solar noon. Values shown are mean and standard error of the mean.

Appendix Table 3. Description of red clover floral maturity index.

Index	Phenological stage
1	From youngest floral meristem (visible bud) to fully expanded inflorescence with unopened florets.
2	Beginning of flowering: few opened florets.
3	About 50 % of opened florets.
4	Majority of opened florets.
5	Beginning of seed set: few desiccated florets.
6	About 50 % of desiccated florets.
7	Majority of desiccated florets.
8	Light brown colored heads. Green, immature seeds.
9	Brown intermediate colored heads. Yellow colored seeds.
10	Dark brown colored heads. Majority of brown, mature seeds.

Appendix Table 4. Seed bearing flowers (SBF) at harvest time in 1990 and 1991, and plant density after the second-year crop for six red clover seed irrigation treatments at Hysop Farm, Corvallis, OR.

Treatment	Seed bearing flowers - 1990	Seed bearing flowers - 1991	Plant density 1991†
	---------- no. m^{-2} ----------		%
NS	860a‡	623a	82.5a
HH	895a	784a	79.8ab
H0	885a	736a	80.0ab
0H	1148a	684a	84.1a
0L	960a	728a	85.9a
C	965a	736a	74.9b

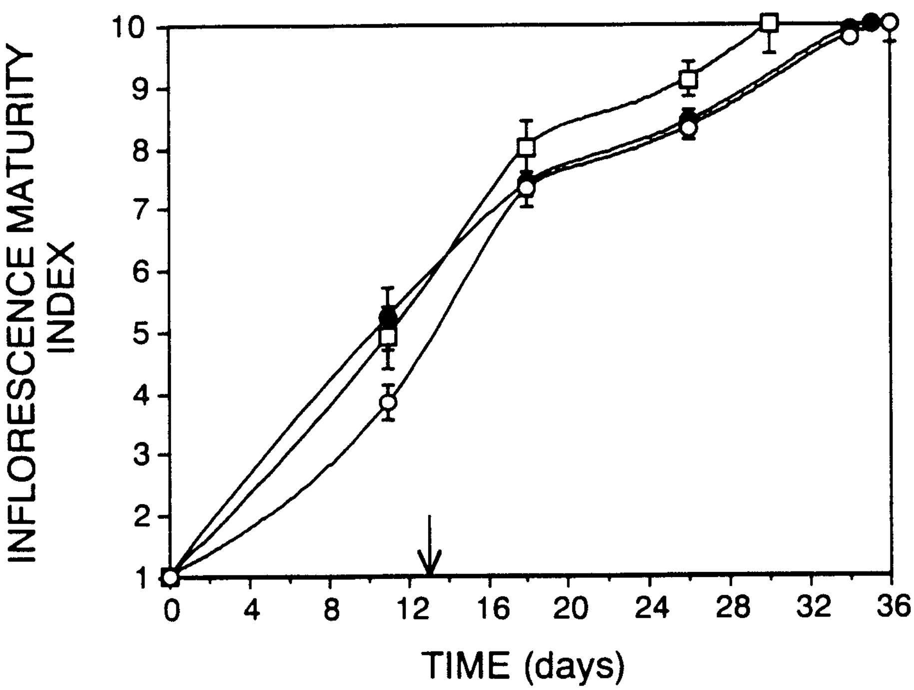
†Estimated as % ground coverage.

‡Within columns, means followed by a different letter are significantly different according to LSD test at $P \leq 0.05$.

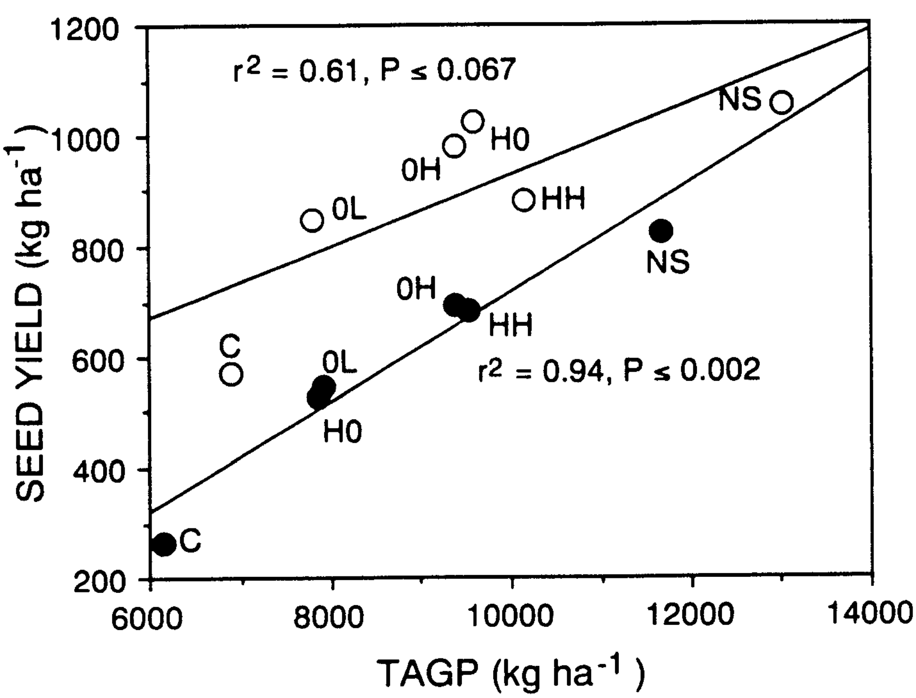
Appendix Table 5. Crop water stress index (CWSI) values for white clover seed control plots with 0, 25, 50, 75 and 100% of mature and flowering heads sequentially removed on DOY 200, 207, and 214 at Hyslop Farm, Corvallis, OR in 1991.

Day of year	Inflor-escences at anthesis/ at pod-filling	Percent of heads removed				
		0	25	50	75	100
	no. m^{-2}	CWSI†				
200	192/100	0.28±0.01	0.28±0.01	0.29±0.01	0.29±0.01	0.29±0.01
207	270/80	0.43±0.01	0.45±0.01	0.43±0.01	0.44±0.01	0.45±0.01
214	235/331	0.77±0.02	0.78±0.02	0.76±0.02	0.78±0.02	0.75±0.02

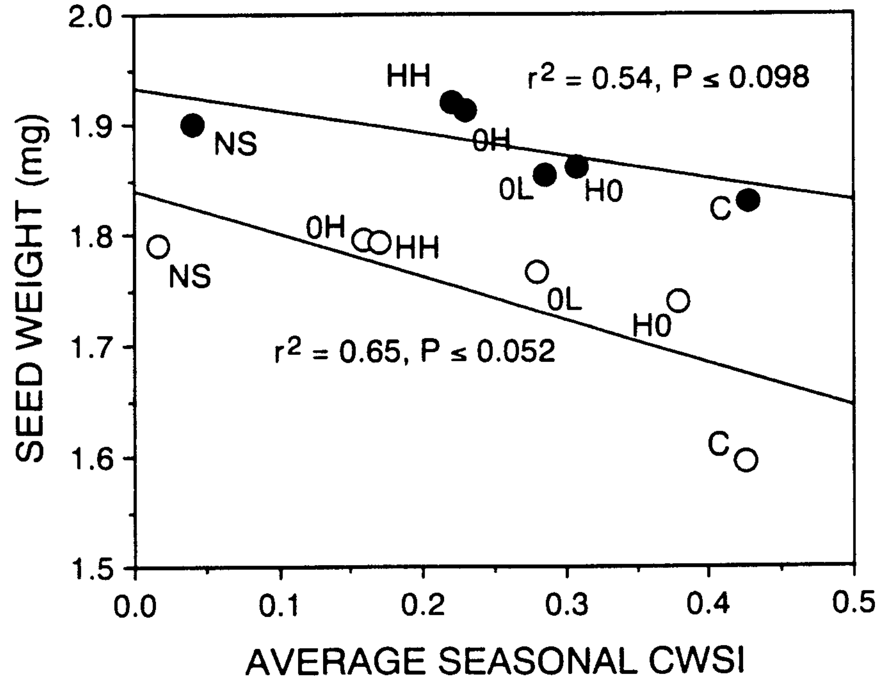
†The non-stressed baseline used was: Tc - Ta = 0.58 - 1.67*VPD, where canopy (Tc) and air (Ta) temperatures are in °C and vapor pressure deficit (VPD) is in kPa. All measurements were made under cloud-free conditions within 2 h after solar noon. Values shown are mean and standard error of the mean.



Appendix Fig. 1. Time-course development of individual inflorescences from bud (1) to dark brown (10) stages for red clover seed irrigation treatments NS (o), 0H (●), and C (□) at Hyslop Farm, Corvallis, OR in 1990. The arrow on the horizontal axis indicates irrigation time in 0H treatment. Vertical bars indicate standard error of the means.



Appendix Fig. 2. Seed yield (SY) as function of total above-ground phytomass (TAGP) for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (O) and 1991 (●). Treatments are indicated beside every data point.



Appendix Fig. 3. Effect of average seasonal crop water stress index (CWSI) on seed weight for six red clover seed irrigation treatments at Hyslop Farm, Corvallis, OR in 1990 (O) and 1991 (●). Treatments are indicated beside every datapoint.