effects of temp have been noted for many species (8, 9, 22) including blueberry (3). Rather, increasing davlength correlated better with decreasing hardiness, also the effect of temp did not remove any additional variation from the equation, and subsequently was deleted. Another explanation is that temp is acting as the trigger for a chain reaction resulting ultimately in dehardening, once the temp rises to a certain level. dehardening starts. Then the dehardening rate remains independent of temp fluctuation within a certain range, in such a case temp would not correlate with hardiness on a per date basis as well as photoperiod (8).

The potential value of hardiness equations is great. Of basic importance are the models, hardiness prediction being a function of several factors not just one. Multiple regression provides a method to study these relationships better. There are several practical applications such as site selection or screening for hardy seedlings. After several years' observations it should be possible to get constant values for partial regression coefficients of significant environmental and phenological components. Then known values of phenological components of commercial cultivars can be put into the equation for determination of survival potential at a site. The breeder can make these measurements on seedlings which will predict their hardiness. From our experience moisture content and bud size measurements are easier to make than any freezing methods currently in use.

Work is being continued in our program to find the constant values for the partial regression coefficients. In the equations, in this paper, the coefficients may not remain constant from year to year. This must be considered as a concern in evaluating the prediction equation's utility at this time.

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## In Vitro Reproductiveness of Asparagus Stem Segments with Branch-shoots at a Node<sup>1</sup>

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Abstract. More rooted Asparagus officinalis L. plantlets were obtained in vitro from stem segments with 3 or more branch-shoots than from those with 1 or 2 branch-shoots; those without branch-shoots produced the fewest plantlets.

We reported a technique and procedure for vegetatively propagating asparagus plants in vitro (1, 2). Explants for culture were obtained from nonbranching stems of aseptically-cultured stock plants. When

<sup>2</sup>Research Associate and Horticulturist, respectively, Department of Horticulture. Acknowledgement is given the Washington Asparagus Growers Association for grant funds supporting these studies. stock plants were cultured for more than 1 month, branching usually occurred on the apical portion of stems killed back by heat from overhead lights; occasionally they developed on uninjured stems (Fig. 1A, B). After 3 months 54% of the stems had branched, averaging 3.2 branches per stem. When a multiple branch-shoot formed an enlargement at a node, essentially an aerial crown resulted at the base of the shoots. In a few cases aerial roots were produced (Fig. 1B). This suggested that these structures may have a generative capacity that could be used as another source for developing plants in asparagus tissue culture. The objective was to determine if there were differences in developing plantlets in vitro between stem segments from stock plants with and without branch-shoots.

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Fig. 3. Asparagus shoots from 5 types of stem segments after 3 weeks of development. 1 -one-bud segments from branching stems, 2 - segments with 1 branch-shoot, 3 - segments with 2 branch-shoots, 4 - segments with 3 or more branch-shoots, 5 - one-bud segments from non-branching stems.

Table 1. Shoot and root development *in vitro* on stem segments from asparagus stock plants with and without branch-shoots.

Types of stem segment	% shoots emerged after 3 wk	No. of shoots after 3 wk	Length of shoot (cm) after 3 wk	% rooted after 9 wk
Branching stem				
1 (1 bud)	65.0c <sup>z</sup>	$2.1c^{Z}$	3.6c <sup>z</sup>	11.1c <sup>z</sup>
2 (1 branch-shoot)	81.3b	3.1b	4.3b	34.5b
3 (2 branch-shoots)	82.7b	3.3b	4.6b	36.0b
4 (3 or more branch-shoots)	89.5a	4.5a	5.1a	49.1a
Non-branching stem				
5 (1 bud)	81.0b	3.0b	4.4b	38.8b

<sup>2</sup>Mean separation in column by Duncan's multiple range test, 5% level. Data was transformed to angles for test of significance.

Only healthy branching stems were excised, and weak-growing apical portions of the stems were discarded. The remaining portion was cut into segments. Each segment included 1 node with a bud or 1 or more branch-shoots. There are 4 types of segments (Fig. 2): 1) those with 1 bud; 2) those with 1 branch-shoot; 3) those with 2 branch-shoots; and 4) those with 3 or more branch-shoots at a node. A 5th type included 1-bud segments taken from the middle and basal portions of vigorous and moderately vigorous non-branching stems.

Five segments of a type were placed in a 125 ml flask which contained 50 ml of MMS medium with 0.1 ppm NAA and kinetin. Flasks were placed in a growth chamber as described previously (1, 2). The experiment was repeated 6 times with 4 to 5 flasks containing segments of each type.

Bud growth of explants began in 3 days regardless of segment type. In the 2nd week, most of the surviving type 1 explants had produced a shoot and a few had produced 2 shoots. Most of the type 2, 3, 4 and 5 explants had produced 2 or 3 shoots. After 3 weeks, shoot emergence was 65.0% for type 1,

89.5% for type 4 and average 81.7% for the other 3 types (Table 1, Fig. 3). The no. of shoots developed from type 4 explants was greater than those from the other types. No difference in shoot production was found among types 2, 3, and 5. Type 1 averaged 2.1 shoots per surviving cultured segment. The length of shoots was 5.1 cm for type 4 and 3.6 cm for type 1 segments. Shoots from type 2, 3, and 5 segments were 4.3-4.6 cm in length.

Roots formed in 3 weeks on type 4 explants; in 4 weeks on types 2, 3, and 5; and in 8 weeks on type 1. After 3 weeks, 49.1% of the explants from type 4 segments had formed plantlets with shoots and roots (Table 1). There were no differences in root formation among explants from segment types 2, 3 and 5. Those from type 1 segments produced fewer rooting plantlets.

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Fig. 1. A. Branching stems on 2-month-old asparagus stock plant. B. Aerial crowns (ac) and aerial roots (ar) on 3-month-old stock plants.

The stock plants were grown for 2 to 3 months in 500 ml flasks containing 100 ml of modified Murashige and Skoog's medium (MMS) (1) with 0.1 ppm  $\alpha$ -naphthaleneacetic acid (NAA) and 6-furfurylamino purine (kinetin).



Fig. 2. Four types of stem segments from branching stems of asparagus stock plants.
1 – one-bud segments, 2 – segments with
1 branch-shoot, 3 – segments with 2 branch-shoots, 4 – segments with 3 or more branch-shoots.