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RESEARCH ARTICLE

Phenological stages of willow (*Salix*)

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Abstract

The practice of uniform recording of biological plant growth stages or events has long been practiced in agricultural production. In this study the BBCH (Biologishe Bundesanstalt, Bundessortenamt and CHemical Industry) code has been applied to four precocious species of willows to define growth stages important to this group. The studied taxa represent varieties of potential importance in the Floral Industry. A new BBCH code is proposed where the annual cycle of willows is divided into clearly recognisable and easily distinguishable developmental phases which include eight principal stages, 30 secondary stages and six mesostages. Photographs illustrate the physical appearance of select stages. This proposed BBCH code shows a unified approach which may be applied to a large number of *Salix* species.

Introduction

Phenological data are important for many aspects of agricultural production as they provide basic information on crop requirements at certain times which are essential to achieve sufficient yield and sustainable management (Chmielewski, 2003). Phenological observations help growers to monitor plant development and determine the optimal time for various cultural practices such as irrigation, fertilisation, harvest time and crop protection.

In order to monitor phenological development effectively and to make comparable observations, it is imperative to accurately define the phenological or growth stages for each crop. Growth stages include points in time which are both biologically important to the plant, as well as significant to human beings. Various approaches to the recording of plant growth stages exist including both numerical and alphanumerical systems (Chmielewski, 2003; Zadoks *et al*., 1974). The BBCH (Biologishe Bundesanstalt, Bundessortenamt and CHemical Industry) is a coding system which may be applicable to all plant species providing uniform recording of plant growth stages (BBA, 2001). The BBCH code specifies details for major crops including dicotyledons and monocotyledons, and addresses development particular to various crops, such as growth from vegetative parts or organs, and stages unique to grasses and perennial plants (Hack *et al*., 1992). Currently, scales exist for a number of woody species such as persimmon (García-Carbonell *et al*., 2002), coffee (Arcila-Pulgarín *et al*., 2002), pomegranate (Melgarejo *et al*., 1997), quince (Martínez-Valero *et al*., 2001), loquat (Martínez-Calvo *et al*., 1999) and peach (Mounzer *et al*., 2008). Recently, a revised uniform scale specific to woody plants was proposed which reflects special growth attributes such as sylleptic and proleptic growth patterns, monopodial or sympodial growth, as well as various developments for deciduous, evergreen or needle bearing species (Finn *et al*., 2007).

Salix L., a woody genus of near-global distribution has been grown as a cash crop since 1800 (Stott, 2001). Numerous uses of willow include its historical cultivation for basketry and other crafts, for the production of charcoal and fuel, as landscape specimens, as ornamental cut stems for the Floral Industry and, more recently, for environmental applications and larger scale bio-energy production (Kuzovkina *et al*., 2008). The long traditions of willow cultivation have resulted in greater understanding of willow biology. However, no detailed information on *Salix* growth stages describing its annual cycle of development has been proposed.

This study was implemented to characterise the phenological growth stages of *Salix*, with emphasis on four taxa of potential importance to the Floral Industry. The objectives of this study were to identify the principal and secondary growth stages, as well as mesostages for willow taxa, and to apply the numerical coding of the BBCH general scale to the identified growth stages.

Willow growth

The genus *Salix* consists of approximately 330–500 woody dioecious species represented by various life forms – trees, shrubs, prostrate plants and ground covers (Argus, 1997). Willows have separate generative and vegetative buds that are covered with a single scale. Flowers are borne in early spring–early summer on mostly erect catkins, or aments. Each flower in the inflorescence is subtended by a floral bract. A staminate flower contains 2 to 12 stamens and a pistillate flower contains a pistil with mostly two-lobed stigma. Seeds of *Salix* are very small and have limited longevity. They are shed 3 to 8 weeks after pollination and seeds of many species germinate immediately on exposed moist surfaces.

One- or two-year-old seedlings retain the terminal bud, and later all species exhibit sympodial growth. Willows do not form terminal buds, but at the end of the stem elongation, the length of stem apex is reduced in size, followed by the abortion of shoot tips. New growth in spring begins with the development of axillary buds from the preceding year.

Nonconcurrent phenology for flowers and leaves is common in willows, and a few patterns of annual development are recognised. In precocious species, flowering takes place before leaves emerge; in subprecocious species, generative budburst and anthesis occur just before the emergence of leaves; in coetaneous species, flowering and leaf emergence take place simultaneously (Dickmann & Kuzovkina, 2008).

Most willows easily form root primordia on stem nodes and root, and in commercial production are typically propagated by cuttings, or dormant shoots. Another distinctive feature of many willows is their ability to resprout from totipotent cells at old nodal areas, such as stumps or stools, and the repeated harvesting of stems (coppicing) is commonly practiced in willow production.

Materials and methods

Field plots were established at the Research Farm of the University of Connecticut located at Storrs, Connecticut: 41° 81 ′N, 72° 26′ W; USDA hardiness zone 6, sandy loam soil, annual precipitation average 1311.66 mm. Four *Salix* taxa for this study were included: *S. acutifolia* Will. (pointed-leaf willow; section *Daphnella* Seringe), *S. gracilistyla* Miq. (Japanese pussy or rosegold willow; section *Subviminales* Schneider), *S.* 'The Hague' (a possible hybrid of *S. gracilistyla* and *S. caprea* L.) and *S. koriyanagi* Kimura (section *Helix* Dumont). These varieties are cultivated for floral stems with showy catkins (Armitage & Laushman, 2003; Kuzovkina & Quigley, 2004; Greer & Dole, 2009). *Salix acutifolia*, *S. gracilistyla* and *S. koriyanagi* were propagated from a single male clone each and *S.* 'The Hague' was propagated from a single female clone. Ten specimens of each taxon were planted from cuttings in spring 2006. Data collection and monitoring began in January 2007 and were recorded through December 2008. Observations were recorded every 3 days from January to April to provide sufficient data on the floral development and on a weekly basis from May to December.

According to the BBCH scale the phenological observations were organised into principal, secondary and mesostages of plant development, where principal growth stages were assigned values of 0 to 9, corresponding to major biological events beginning with stage 0 for germination/sprouting/bud development, continuing through stage 9 for senescence/beginning dormancy. Secondary growth stages described shorter developmental stages occurring within a principal growth stage and were recorded as a second digit to provide an in-depth double digit description of development. Principal and secondary stages were based upon the development of the main stems. If a more detailed description was necessary for the main stems, leaves or side shoots, three digit mesostages were assigned.

Description of the phenological growth stages of four *Salix* species

Principal growth stage 0: Dormancy

00 Buds covered by closed scales

This stage is achieved after leaf drop. There are no visible signs of vegetative growth or floral development. Floral buds which differentiate the previous summer have achieved their winter colour. *Salix gracilistyla* bud scales appear glossy red (Fig. 1).

Principal growth stage 1: Leaf development on main stem

10 Vegetative bud burst

Vegetative bud burst is reached when the first green tissue becomes visible, or leaf tips are protruding from the stems or stools. If the stems were coppiced during the dormant season, the stage is recorded when stumps show shoots with first leaf initials on the nodes at the base of a plant (Fig. 1).

11 Leaf expansion

Leaf lamina is fully expanded. Tip or petiole reflexes.

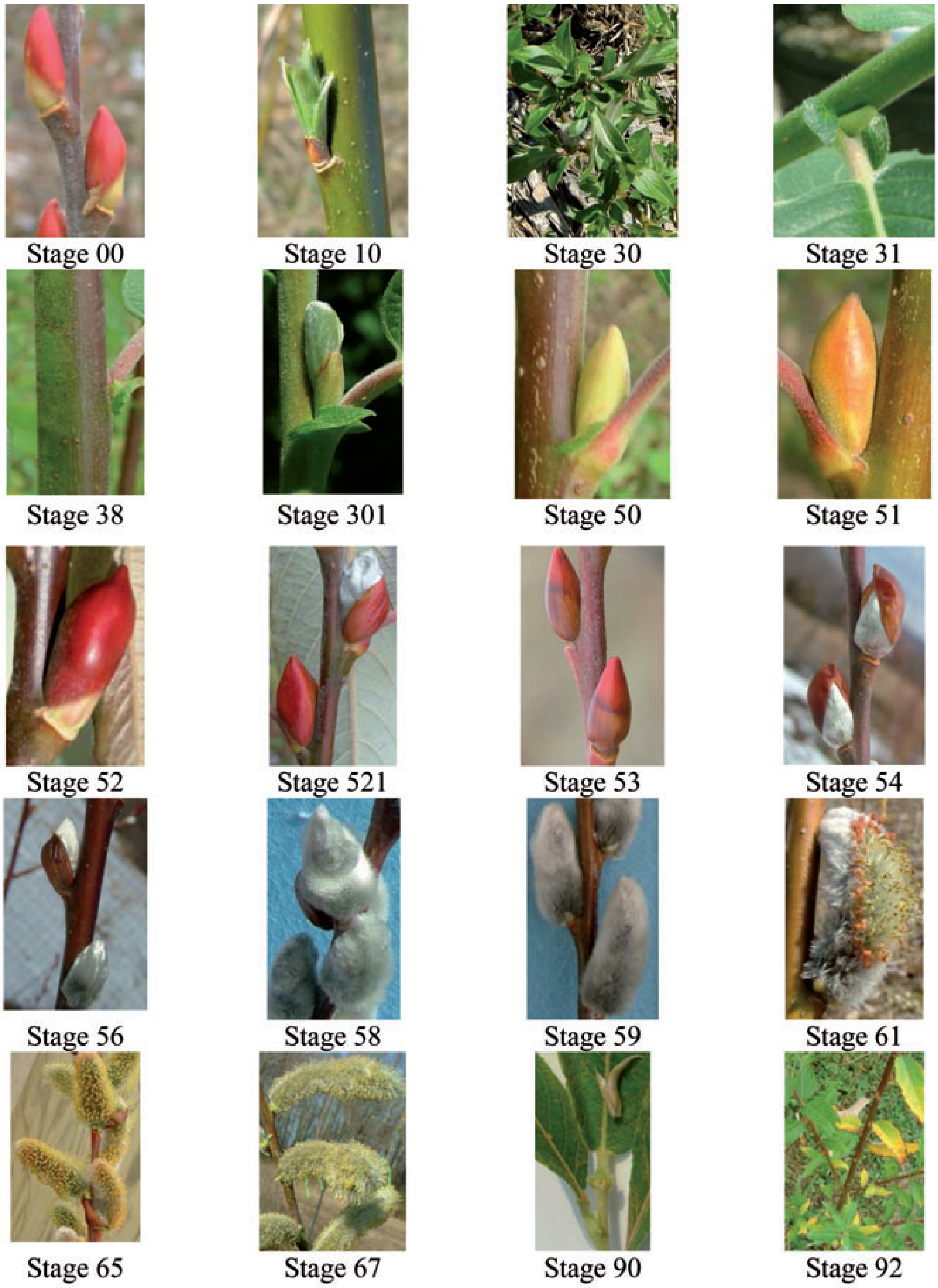

Figure 1 Phenological growth stages of *Salix gracilistyla.*

Principal growth stage 3: Stem elongation

This growth stage refers to the development of proleptic branches.

30 Beginning of flushing period and stem elongation

Growing shoot tips and axes become visible (Fig. 1).

31 Formation of axillary buds

Axillary buds are visible when the petiole is reflexed from the stem (Fig. 1). During the first few weeks after leaf burst, leaves are fragile and may be broken off while bending them back looking for the axillary bud. Generative and vegetative buds are not distinguishable from one another.

32 The increments in stem length reached 25% of its final length

35 The increments in stem length reached 50% of its final length

37 The increments in stem length reached 75% of its final length

38 Stem colouration begins

Soft tissues of annual stems change colour as the growing season progresses (Fig. 1). Colour advancement on stems varies greatly on the same plant and depends on sun exposure and branch position. For example, *S. gracilistyla* stems exposed to sunlight become purple/tan beginning at the base of the plant (Fig. 1). Changes in bark colouration may be because of anthocyanin accumulation, manifesting as changes in stem colouration from green to reddish, signalling early stages of the cold hardening process (Lennartson, 2003).

39 Bright stem colouration

The stage is manifested by maximum colour development on stems.

Mesostages: Development of sylleptic branches

301: Beginning of flushing of sylleptic branches (Fig. 1)

311: Axillary buds become visible in axils of leaves on sylleptic branches

321: Axillary bud development into third order branches

331: Visible differentiation of generative buds on sylleptic branches

341: Shoot tip abortion on sylleptic branches

Principal growth stage 5: Inflorescence emergence

50 Differentiation of generative buds

As inflorescence initiation and enlargement continue in summer, larger generative buds become clearly distinguished from smaller vegetative buds (Fig. 1). This is called 'green bud' stage as buds have not yet begun to flush with colour. In studied taxa, large generative buds occurred mostly on the upper portion of the stem, while smaller vegetative buds occurred below.

51 Colouration of generative bud scales

Buds scales changing colour from green to reddish, brownish or yellowish depending upon species. In *S. gracilistyla*, generative bud scales appear to blush or redden on stems which are exposed to sunlight (Fig. 1). This stage was called 'bud blush'.

52 Maximum colouration of generative bud scales

Generative bud scales have reached their maximum colour or 'winter colour' (Fig. 1).

521: Premature generative bud burst (Fig. 1)

Occasional generative bud burst takes place; bud burst occurs even before leaf abscission has completed during the same year in which the inflorescences form. This stage appears sporadically or completely, depending on taxa and weather conditions. Bud scale burst frequently occurs on the adaxial side where the catkin appeared to protrude or push through the scale (Fig. 1). This differs from the normal development of winter bud burst, when the bud scale splits along surfaces other than only the adaxial and/or detaches from the branch at the proximal end. Premature bud burst can be attributed to mild weather conditions (Newsholme, 1992).

53 Generative bud swelling

Generative bud swelling is the first visible stage indicating the beginning of inflorescence expansion. Generative buds form during the preceding year and continue development throughout the winter, resulting in gradual expansion of the inflorescence. The content of generative buds remains within the bud scale. During this stage generative bud scales may change colour, surface (glabrous to pubescent) and translucence (opaque to papery/dry) indicating a progression from dormancy to flower development (Fig. 1).

54 Beginning of generative bud burst

Bud burst signifies the next stage of inflorescence expansion. As the inflorescence gradually enlarges it pushes off the bud scale, and the light inflorescence tissues become visible through splitting bud scales (Fig. 1). This event is recorded when bud scales crack on 50% of catkins. Flower bract hairs remain flattened or pressed to the main axis of inflorescence.

55 Advanced generative bud burst

Generative bud scales crack on 95% of catkins.

56: Generative bud scales drop on 50% of catkins

Beginning of generative bud scale drop. This stage is called 'paint brush stage'; because bract hairs were visible, they did not become erect on the inflorescence main axis (Fig. 1).

57 Generative bud scales drop on 95% of catkins

Advanced generative bud scales drop.

58 Beginning of catkin elongation

Fifty per cent of inflorescences elongate noticeably beyond bud scales. Bract hairs become visible and rise from the main inflorescence axis, giving a 'furry' appearance. This is the classic 'pussy willow' image (Fig. 1). This stage is called 'ornamental harvest stage' as it signifies the economically important stage for floral production at which time catkins are at peak ornamental value.

59 Inflorescence maximum expansion

Ninety-five per cent of inflorescences reached maximum length. This is called the 'post-peak' stage as stems are considered beyond the time for commercial harvest (Fig. 1).

Principal growth stage 6: Flowering

61 The onset of flowering

First sporadic flowers appear when stamens or stigmas become distinguishable on 10% of flowers (Fig. 1).

65 Full flowering

Anthesis reaches 50% of inflorescences. Stamens with bright pollen and receptive stigmas become visible (Fig. 1).

67 Inflorescence wilting

Fifty per cent of inflorescences wilt (Fig. 1).

69 Inflorescence dropping

Fifty per cent of inflorescences detach from the stem. This stage is only applicable to male plants. Male aments usually fall off soon after flowering, while female plants develop dehiscent capsule fruits and the aments drop after seed ripening (stage 89).

Principal growth stage 7: Fruit development

79 Capsule fruits final size

This stage is applicable to only female plants when a pistil (ovary) in a flower swells and reaches its maximum size.

Principal growth stage 8: Fruit ripening and seed dispersal

89 Capsule fruits fully ripe and seeds disperse

This stage is apparent when capsules dehisce and cottony masses of seeds are visible and are detaching from a plant. Female inflorescences drop soon after seed ripening and dispersal.

Principal growth stage 9: Senescence, beginning of dormancy

90 Shoot tip abortion on main stem

Termination of shoot elongation is recorded at the time of the abortion of shoot apex on the main stem (Fig. 1).

92 Leaf discolouration

Fifty per cent of leaves discoloured (Fig. 1).

95 Beginning of leaf drop

Fifty per cent of leaves dropped.

97 Completion of leaf drop, beginning of dormancy

Hundred per cent of leaves dropped.

Discussion

Accurate and detailed descriptions of plant growth phenostages are necessary for comparisons between different taxa, year to year assessments of development for each taxon, for phenostage predictions and for identification of timing of cultural practices (Chmielewski, 2003). This study provides further indication of how the BBCH code may be modified to suit individual woody taxa. Many of the *Salix* stages (0, 1, 3, 5, 6, 7, 8 and 9) are concurrent with the BBCH code and with a modified code for woody species (Finn *et al*., 2007). Principal stages 2 ('Formation of side shoots, tillering') and 4 ('Development of harvestable vegetative plant parts'), described by the BBCH code, have been omitted by Finn *et al*. (2007) and are not included here, as they are not applicable to *Salix*. Interestingly, sylleptic branching, common for tropical and a few temperate species (Finn *et al*., 2007), was observed in all studied willow taxa, and the stages of sylleptic branch development are presented as five mesostages under the principal growth stage 3 'Stem elongation'. At certain times a few overlapping stages

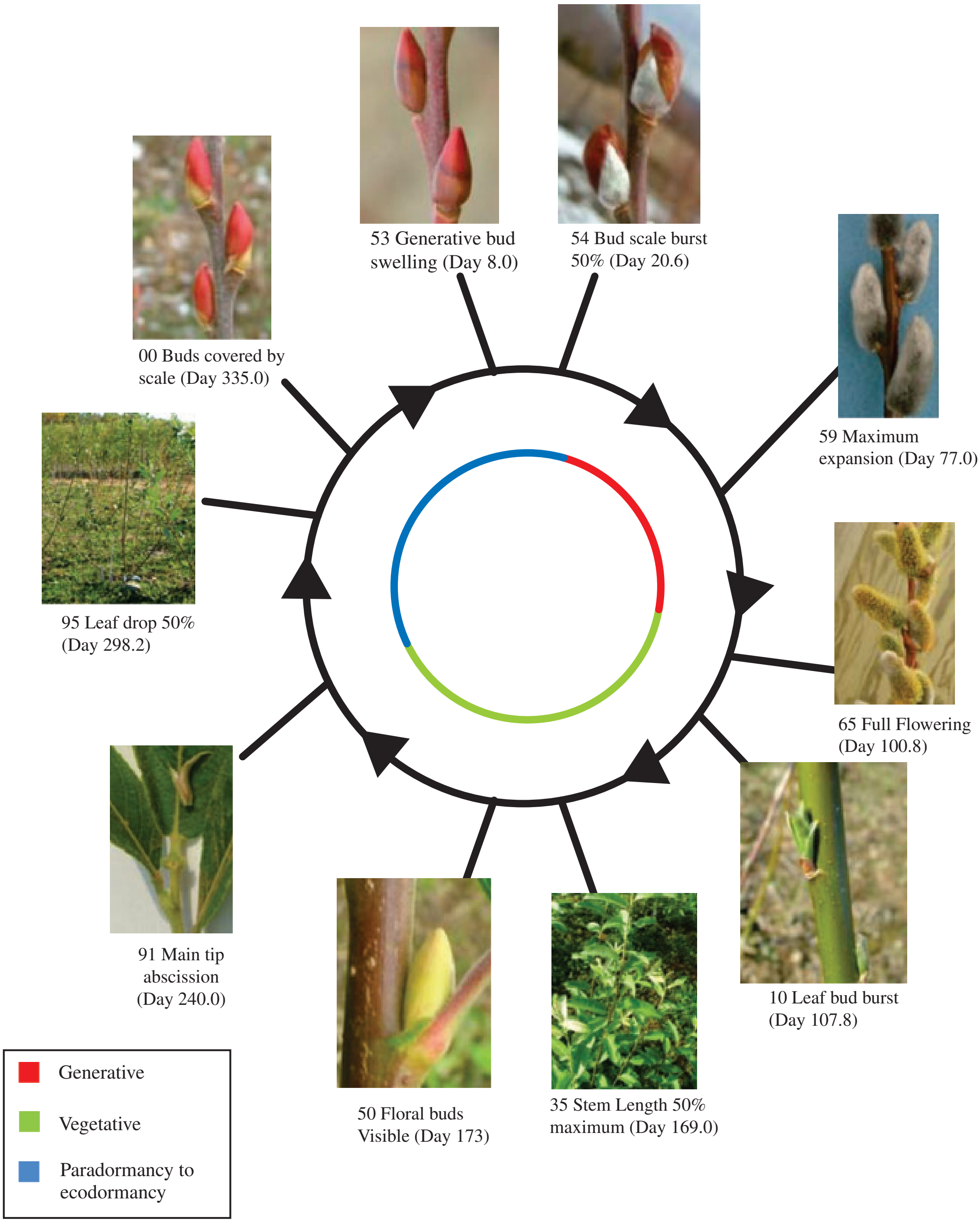

Figure 2 An annual cycle of a precocious species *S. gracilistyla* with the mean day of year when the selected stages were recorded in 2007 and 2008. The annual cycle is divided into general periods of development which include generative and vegetative development, as well as the period of dormancy preceding the generative development.

were recorded on specimens. For grading these parallel events, a few stages can be recorded using slashes, for example 38/341.

All observed taxa – *S. acutifolia*, *S. gracilistyla*, *S.* 'The Hague' and *S. koriyanagi* – exhibited precocious development. A yearly cycle of *S. gracilistyla* is depicted graphically in Fig. 2. It illustrates that in case of precocious development, the phenostages numbered in accordance to the BBCH code, develop non-sequentially, when generative budburst and flowering (principal stages 5 and 6) occur sometime before leaf development and stem elongation (principal stages 1 and 3). In case when stems were coppiced during the dormant season, vegetative growth starts from a few buds at the base of a plant but the principal growth stages 5–8 will not occur because the generative buds were removed from the stools during coppicing.

The scope of this study provided the opportunity to describe the developmental stages for a number of taxa with emphasis on details important for florists. Certain stages, such as floral bud development, were described in detail because of their importance to *Salix* cut-stem producers. Some stages, such as manifestation of bud or stem colouration have not been directly addressed by the BBCH code nor by the code for woody plants. Nonetheless, they were described in this study because of their importance for ornamental cut-stem producers. Observations of changes of bud scales leading to inflorescence development will help producers to prepare for harvest which takes place shortly after bud burst stage.

The proposed scale should be modified slightly for other species to reflect the various subprecocious and coetaneous developmental cycles as various taxa may differ in their phenostage timing, duration and physical appearance. As the extent and significance of *Salix* cultivation are escalating, it is expected that understanding of *Salix* growth stages will be applicable for various production systems and will have broader implications for crop advancement in the future.

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