A FEW OBSERVATIONS ON DOUBLE SIGMOID FRUIT GROWTH

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Abstract:

Context and purpose - Many fleshy fruit, including the grape berry, exhibit a double-sigmoid growth (DSG) pattern. Identification of the curious DSG habit has long been attributed to Connors' (1919) work with peaches. Connors' description of a three-stage pattern consisting of two growth stages (Stage I and Stage III) separated by a rest period (Stage II) has become textbook material. The growth of grapes was described similarly by Winkler and Williams (1936), Nitsch et al. (1960), and most subsequent authors. Prior to Connors, grape berry development was described as a two-stage process, in French *periode herbacee* and *periode maturation*, but this description refers to fruit ripening and has little or nothing to do with growth.

Material and Methods - A review of grape literature reveals that the characteristic DSG habit was reported several times prior to Connors' discovery in peaches. Analyses of berry size, turgor, firmness, and composition during Stage II and into Stage III are interpreted in the context of the growth habit.

Results - It will be argued that one researcher in particular, Carl Neubauer, should be credited with the discovery of DSG and its description as a three-stage phenomenon in fleshy fruits. It is widely reported that DSG in fleshy fruit is a consequence of within-fruit partitioning (to endocarp or seed rather than pericarp/flesh). However, DSG is observed in berry dry weight and in seedless berries, which negate the common explanations. Thus, one hundred-fifty years later, the nature of double-sigmoid growth is still not understood. It is the resumption of rapid growth that is most curious. Various lines of evidence from our studies suggest that a suite of physiological changes during Stage II lead to the transition from Stage II lag phase to Stage III growth, paradoxically implicating a role of low cell turgor. Turgor declines and berries soften during Stage II. These changes occur in conjunction with increased apoplastic solutes and ABA, followed by increased sugar influx and upregulation of cell wall loosening enzymes. Because growth increases in the face of very low turgor, Stage III growth is hypothesized to result from cell wall loosening or even wall degradation without addition of new wall material.

Keywords: berry, fruit, growth, water relations, turgor, cell wall, ABA

1. Introduction

Berry size is an important quality parameter, but the growth process by which size is realized has been given limited attention compared to the ripening process. Growth in most organs and organisms occurs in an approximately sigmoid pattern, but in some fleshy fruit, including the grape berry, a double-sigmoid growth (DSG) pattern is observed. In horticulture, credit is generally given to Charles Connors (1919) for discovering the DSG phenomenon during observations on several peach varieties at the New Jersey Agricultural Experiment Station in Vineland, NJ. According to Connors, peach growth occurs in three stages:

> "Stage 1. Rapid development of the fruit apparently due mainly to increase in the size of seed part.
> Stage 2. Rest period during which the seed is formed and the stone becomes hard.
> Stage 3. Period of rapid growth of flesh to maturity beginning 4 to 5 weeks before ripening time."

The double-sigmoid growth pattern is observed in many other fleshy fruit including all stone fruits, fig, blueberry, olive, etc.. The Connors scheme has been invoked by most horticulturists who have studied growth in these fruits (e.g., Tukey 1936; Crane 1964; Pavel and DeJong 1993; Conde et al. 2007), becoming the 'textbook' characterization of the DSG pattern (e.g. Jackson and Looney 2011). Many authors use it without reference – i.e. as implicitly understood by the readers. In grape research, however, Connors' work is seldom recognized, and Bryan Coombe is often cited as the source of the three-stage description of growth. Coombe (1976) himself acknowledged Connors' "classical" description, and concurred with Connors notion that the length of Stage II determined the earliness of a variety. In addition, identification of Stage II is fundamental to some yield prediction protocols. Thus, the berry growth pattern is of practical importance as well as inherently curious.

Nevertheless, Connors' scheme has been somewhat controversial in the grape literature (e.g., Coombe 1976, Friend et al. 2009, Coombe and McCarthy 2000), with some authors preferring a 2-stage description. In contrast to the stone fruit, it is not clear how we came to recognize the DSG pattern in grapes. Therefore, the history of berry growth research was investigated.

2. Material and methods

The work presented herein is the result of historical research into the understanding of berry growth.

3. Results and discussion

3.1. *Double-sigmoid fruit growth was revealed first in grapes.*

Connors drew his conclusion, "The growth of fruit of peaches is quite definitely divided into three stages." from tabled data on the size of peach fruits of several varieties taken several time during a single season (Connors 1919), an example of which is plotted in Figure 1. In searching for the history of similar measurements in grape, I worked backwards from Coombe's (1976) excellent review article on fleshy fruit development. I found that sustained research on berry growth began in the 1960s following on the excitement generated by the discoveries of several plant growth regulators. However, DSG in grapes was revealed previously: DSG in cv. Carignane was shown and described as per Connors' 3-stage scheme by Winkler and Williams (1936). DSG was also clearly demonstrated in two varieties by Matsuzaki (1930). Those are the origins of berry growth in Japan and the US, but not the beginning of berry growth studies. In Pratt's (1971), also excellent, review article she refers to Lewis (1910) as the first to plot measurements of berry and cluster growth (Fig. 2). Lewis' fruit growth data, on five varieties grown near Cape Town, South Africa, clearly reveal DSG in each variety, although the rate of growth during Stage II varied among varieties. Also, in contrast to Connors, Lewis did not describe the growth pattern of berries. Lewis may well have been the first to plot berry size during development, but his work seems to have been lost to contemporary students of fruit growth, and Matsuzaki's study is apparently unknown outside of Japan.

Interestingly, the history of research on grape berry growth goes back much further. The earliest record appears to be measurements by Carl Neubauer in vineyards near Wiesbaden Germany in the late 1860s. Figure 3 shows a data set from Neubauer that was reported in *Traite de vinification* by Brunet (1894). Brunet cited Neubauer both for discovering the complex growth pattern, and for describing it in three stages – well ahead of Connors:

> "During the first period, the so-called herbaceous period, the weight of the berry increases, …
> During the second period, weight of the berry remains stationary and the proportion of sugar increases, …
> During the third period, the weight of the grain increases as well as the proportion of sugar, …"

There are at least two other publications showing DSG in grapes prior to Connors (1919) report on peaches:
Ernst Mach (1876) in San Michele, Tyrol; and Dugast and Poussat (1895) working in Algeria. Together with Lewis' compelling data set with five varieties, it is irrefutable that the DSG pattern of fruit growth

was first discovered in grapes, and apparently the three-stage description thereof also originated in grapes.

3.2. *Are there really three stages?*

In the classic textbook General Viticulture, Winkler et al. 1974 state à la Connors: "In all grapes, berry enlargement proceeds through three distinct periods and follows a double sigmoidal growth curve." Yet, some ambivalence about the nature of the berry growth habit and its description has lingered. Coombe (1976) calls the inclusion of a third (Stage II) 'arbitrary', and Friend et al. (2009) refer to Stage II as 'artificial'. A few authors have begun employing a two-stage description; e.g., Coombe and McCarthy (2000) refer to two 'cycles' of growth and to two phases of development – berry formation and ripening.

This is a reversion to the pre-history of berry growth research when insight was derived from taste and appearance. Lewis (1910) failed to see the significance of his growth data that Connors recognized in his peach study, referring only to two 'sections' – before and after veraison. Such was the 19th century zeitgeist of berry development, the '*periode herbacee*' and the second '*periode maturation*' (e.g., Guillon 1905). But some of Lewis' contemporaries at the beginning of the 20th century, at least Raymond Brunet and Pierre Viala, were onto the DSG pattern. Indeed, Viala and P'echoutre (1910) wrote,

> "Of course, two stages are apparent to the eye (and fingers, and mouth): green, hard, sour; and then not green, soft, and sweet. A three stage development is only possible to ascertain with several careful measurements of fruit size, whereas the change of color is impossible to miss." [trans. from original French by Google]

Nothing is gained by transitioning to the two-stage description, but something is lost. Using a description that eliminates explicit recognition of the DSG pattern confounds growth with ripening and distracts from the curious aspect of fruit growth that is the DSG pattern. This mistake may only possible in grape where the resumption of growth and onset of ripening are coincident. For DSG fruit in general, ripening begins after the second growth period is essentially complete. Thus, for most DSG fruits, one cannot combine growth and ripening into a single stage.

The three-stage scheme is a common-sense framework for the study of fruit growth that has been overwhelmingly adopted in horticulture. Although Coombe, McCarthy, Friend and others have depicted the three-stage scheme as having logic problems, none are evident. The three-stage scheme provides no hypothesis as to the nature of DSG; it simply denotes what is self-evident in the time-course of fruit growth – growth, no growth, growth. As such, it is a convention that provides a temporal benchmark for physiological processes, in particular those leading up to veraison, and it facilitates comparisons between treatments, studies, and species with DSG. Thus, due its high utility and wide acceptance, it would be detrimental to isolate grape research from the rest of horticulture in the study of fruit growth.

The common-sense nature of the NCS is made evident by the fact that even among authors who have chosen to adopt a 2-stage model, most use the term 'lag phase' in addition to two cycles, stages, phases, etc. (including Coombe and McCarthy 2000). Indeed, the key aspect in DSG is the lag in growth. Without the lag, nothing is unusual or distinguishing in berry growth. These are separate issues – the growth pattern and what we call it. The pattern of two periods of rapid growth separated by a period of little or no growth just is. Coombe (1980) makes my point: "Terminology. It is usual to describe the development of fruits with double-sigmoid growth curves in three stages I, II III. This is not done here; rather, the fruit is regarded as having two consecutive growth cycles separated by a phase of slow or nil growth." Thus, the issue reduces to word choice.

3.3. Is there a physiological basis to the "lag phase"?

Aside from the choice of description, the question arises whether there is a biology specific to Stage II. Friend et al. (2009) and others explicitly argue that there is no physiological basis on which to designate a Stage II. On the other hand, Coombe reported that the varieties with longer Stage II were later to maturity, and that he selected cv. Doradillo as an experimental system due to its long Stage II. This relationship has been thought to hold for other DSG fruits. If correct, an improved understanding of the biology of Stage II would be important for progress toward modifications of variety earliness/lateness.

The duration of Stage II is variable depending on both genotype and environment, indicating that something separates the two growth periods rather than a hardwired contiguousness. Furthermore, the duration of Stage II in some cases is so long that the pattern appears inconsistent with an interpretation of two contiguous sigmoid growth cycles (e.g., approximately 25 days in Downton and Loveys 1978; Ollat and Gaudilere 1998). According to Coombe (1976), the duration of Stage II varies between 8 and 48 days within cv. Muscat of Alexandria.

There is also old and new evidence of Stage II-specific physiology, including solute accumulation and berry softening. A slow increase in Brix during Stage II has been demonstrated many times, originating perhaps with Dugast (1900) (Fig. 4). This slow increase distinguishes Stage II from both Stage Stage III, preceding the rapid influx of sugars that occurs at veraison and I. The Stage II Brix increase may be related to the concomitant accumulation of apoplastic solutes and decrease in cell turgor that occurs in the developing grape berry (Thomas et al. 2009; Wada et al. 2009). The decrease in pericarp cell turgor during Stage II correlates very closely with measurements of berry softening (Fig. 5) (Thomas et al. 2009). In addition, there is evidence of a lag phase in the expression of pectic methylesterase, a key component of cell wall disassembly. Thus, it is likely that there are multiple Stage II-specific aspects of berry physiology. However, none of these processes has been linked to the diminished growth of the lag phase. Both the decrease in growth during Stage I and the resumption of growth in Stage III are likely to be regulated in cell wall biology because turgor is high when growth slows and is low when growth resumes.

4. Conclusions

Growth in and of itself is clearly worthy of study. Fruit growth should be a field of study, and it should have a history and milestones. DSG growth is unique to fleshy fruit. The Connors scheme, based on peach studies, that is widely used to describe DSG is 100 years old today. However, both DSG and a three-stage description thereof was discovered in grapes much earlier by Carl Neubauer. The proposed reversion to a 2-stage model would isolate grape research from the vast majority of DSG fruit investigations, including its own history of employing the 3-stage scheme. Although there are aspects of grape physiology that are unique to Stage II of the three-stage scheme, the fundamental nature of double-sigmoid growth in the berry remains an enigma.

5. Acknowledgments

The author thanks Professor Leo Gros, Hochschule Fresenius, Wiesbaden, Germany for assistance with research on Carl Neubauer.

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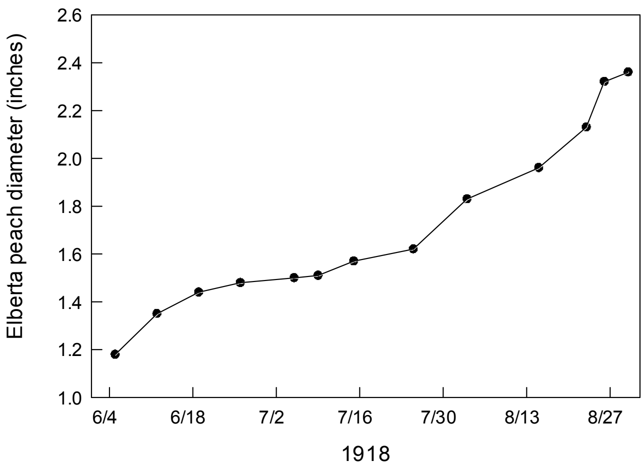

Figure 1: Changes in diameter of Elberta peach fruit during the season. Data plotted are the original data from Table 8 in Connors (1919).

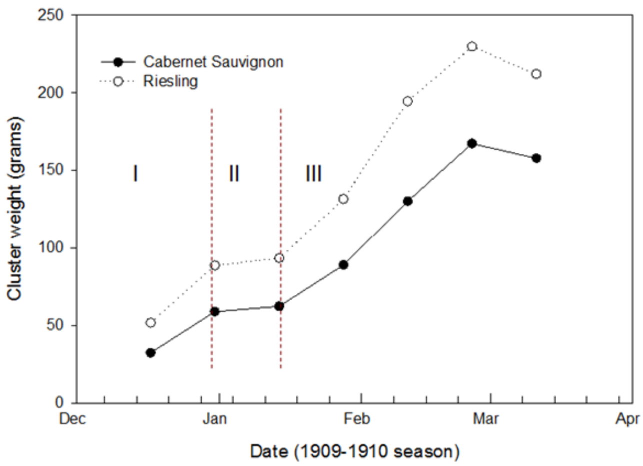

Figure 2: Changes in grape cluster weight of Cabernet Sauvignon and Riesling during the season. Data plotted are from Table I in Lewis (1910).

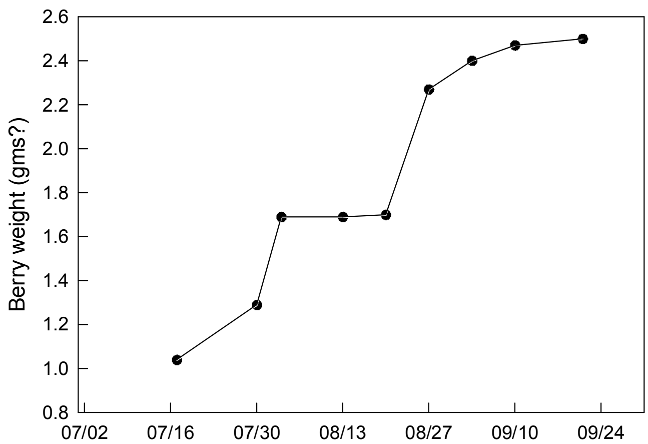

Figure 3: Changes in berry weight during the season. Data are from Brunet (1894), and attributed to Carl Neubauer therein.

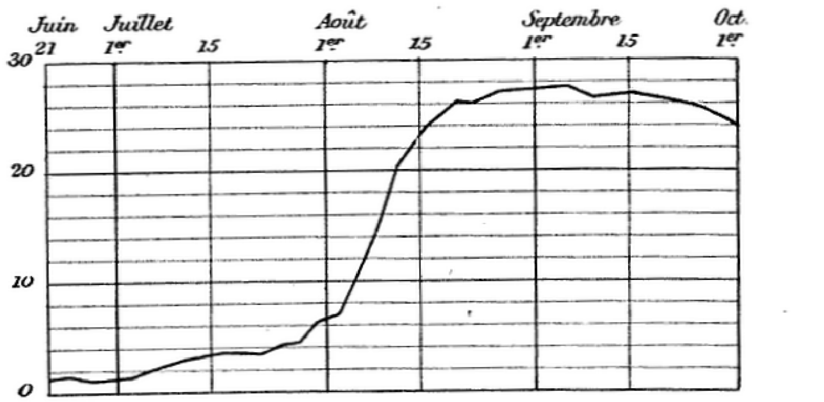

Fig. 5. — Courbe représentant la richesse saccharine du moût, à partir de la véraison.
Une division horizontale correspond à 2 pour 100 de sucre.

Figure 4. An early example of the slow increase in soluble solids that is often observed during Stage II [copy of Fig. 5, p16 in Dugast (1900)].

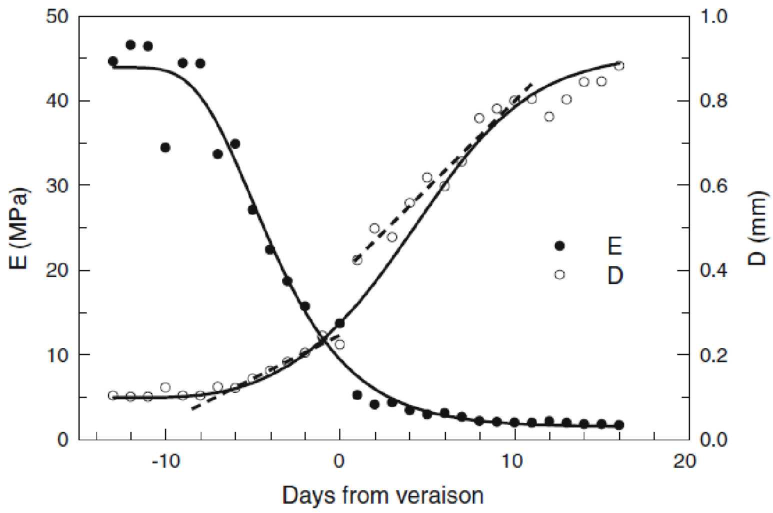

Figure 5. Re-evaluation of berry softening using data from Coombe (1992). Deformability (D) data are Coombe's original data, Elasticity (E) data are recalculated softening using the Hertz equation to correct for changes in fruit size and contact area between flat plates and elastic sphere (berry). The E data show softening commencing during Stage II before veraison. This is earlier than previously recognized and proceeds in concert with decreasing berry cell turgor prior to veraison (see Thomas et al. 2008). Redrawn from Thomas et al. 2008.