

### **Research Article**

## **Pod Production Dynamics and Pod Size Distribution of** *Theobroma cacao* L. Clone CCN 51 in Full Sunlight

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Cacao fruit production dynamics vary from one location to another and are conditioned by the number of pods produced per tree. During cocoa pod development, the strength of the carbon sink varies depending on the demand exerted by the pods, which is proportional to the size. The relationship between cocoa pod production dynamics and size distribution is still poorly understood. Dissecting this relationship is an important step toward further improving cocoa crop management. In this study, the annual yield dynamics and quantity of cocoa pods produced by popular, highly productive, and widespread clone CCN 51 were investigated, based on six size classes observed during its fructification. Growth parameters were determined as weekly increments of pod length and diameter, whereas daily increments were estimated using the logistic Richards model. The fruiting cycle was characterized by the coexistence of fruits of various sizes where the number of pods belonging to each size class changes throughout the fruiting season. Fruit production varied following a seasonal pattern, reaching a maximum of 36 pods/tree, in trees cultivated in full sunlight, of which approximately 55% matured and were harvested. The peak carbon sink demand occurs when the tree pods have the highest numbers of pods. During this period, 65% of the pods had lengths between 5 and 15 cm, which corresponds to the period of the highest pod growth rate. The average length values of the harvested pods were generally below 23 cm and rarely exceeded 7 pods/tree. The Richard model proved to describe accurately the pod growth rates for CCN 51. This represents a promising tool to determine pod growth in other cultivars of relevance for the cocoa industry, which is essential to improve cocoa crop management.

#### 1. Introduction

Cacao (*Theobroma cacao* L.) is one of the most important perennial tropical crops in the world. The seeds (almonds) of its fruits (pod) are used for the production of chocolate. In the period 2021/2023, approximately 4.8 million tonnes of cocoa were produced [1]. Cacao crops are traditionally planted in regions with annual precipitation ranging between 1500 and 1800 mm [2]. However, the use of irrigation

systems and the selection of drought-resistant clones have allowed expanding cacao plantations to drier climates [3, 4], with annual rainfall of 800–1000 mm [5].

Cacao tree ecophysiology has also been studied in a fair number of cultivars and clones, which may be cultivated in shaded or in full-sunlight conditions [6–8]. However, there are still key physiological aspects that remain poorly understood, such as the high proportion of flowers produced vs. the relatively low number of fruits that are harvested. Previous studies indicate that approximately 75% of the fruits originating from fertilized flowers will cease growing 40-50 days after anthesis, remaining in the stage commonly described as cherelle [9]. After a few days, the cherelles turn yellow, necrotize, and may persist in a "mummified" condition during an undetermined period on the tree [10]. This process is commonly known as "cherelle wilt" and has been attributed to a water imbalance in the fruit tissues caused by turgor loss due to xylem occlusion. Losses due to cherelle wilt may be diminished by improving soil quality conditions, mainly by ensuring a regular water supply and providing adequate mineral nutrition through regular fertilization, since both measures have proven to be more effective in reducing cherelle wilt than shading cacao trees [10]. Previous studies indicate that the periods of the highest flowers and new cherelle production coincide with the peak of the rainy season, which also happens to represent the season with the highest rates of aborted pods [11]. The probability of pod abortion is also related to the timing of cherelle formation; for example, cherelles produced at the beginning of the fruiting season have a lower propensity to abort, compared to those produced at the end of the season, since early season cherelles have access to higher concentrations of available photoassimilates, compared to the late season ones, which face heavy competition for the same resources with the maturing reach [12].

The estimated duration of cacao pod growth and development is 150 days, counting from the moment flower anthesis occurs. Once the pods overcome the stage known as cherelle, pod development occurs mainly due to increments of length and diameter during a period of 100-110 days, and pods reach maturity 20-30 days after they have completed their expansion [9]. Nevertheless, detailed studies depicting the number of pods produced and pod growth dynamics during each development stage are lacking. According to Adjaloo et al. [11], the number of small pods (0.5–1.0 cm) greatly exceeds the number of larger ones throughout the year. Each fruit accounts for a carbon sink in itself; however, the strength attained by each sink will depend on both the actual number of pods at a given moment and their development stage. Other facts that need to be evaluated are the timing and the number of pods harvested during each fructification season, since these in both cases are influenced by the type of cultivar, as well as regional variations, depending on the prevailing climate.

The relationship between pod growth rate dynamics and their distribution according to size on the same tree remains unclear. This information could greatly improve crop management strategies, including disease and pest control, fertilization, and pruning practices. Furthermore, it can be used to develop production models adjusted to each cacao cultivar, considering the parameters that presumably have a stronger influence on fruit production dynamics. In this regard, this study aimed to describe the relationship between fruit production dynamics and pod size distribution. Two consecutive years were evaluated for yield, while pod growth and pod size based on the stage of pod development were collected only for 6 months in a cacao plantation composed of the popular clone CCN 51. Here, the popular and widespread clone CCN 51 was selected as a model. CCN 51 is a well-recognized cultivar due to its exceptional adaptability to a large range of different climates, including semiarid ones, thanks to an advantageous set of traits, which include aspects related to its reproductive phenology (long pollen longevity), physiology (osmotic adjustment, high nonphotochemical quenching, and low Cd accumulation), and high productivity (low pod index) [13]. This clone is currently cultivated in Ecuador, Colombia, Perú, and Brasil [5, 14–16]. Our findings allow us to establish the pod's growth curve and predict more accurately the yield of clone CCN 51 when it is cultivated in full sunlight and receiving regular irrigation.

#### 2. Materials and Methods

2.1. Site Description and Cacao Clone. The assay was conducted between 2016 and 2017, in a 146 ha<sup>-1</sup> plantation composed exclusively of the clone CCN 51, which was established on land of the farm Río Lindo Alto, owned by the company AGROTROPICAL, which is located between the towns of Quevedo and El Empalme (1°04'54.32"S and 79°34′37.1″W), at an elevation of 95 m.a.s.l. in Ecuador. The soil is mostly silty loam. The daily air temperature, relative humidity, evapotranspiration, and rainfall values were recorded during the rainy (December through March) and dry seasons (June to October) by the Pichilingue Weather Station of the National Institute of Meteorology and Hydrology (INAMHI). The climate on the Ecuadorian Coast is defined by two well-marked seasons: the rainy season starts the second week of December and lasts until May, and the dry season starts in June and generally extends until early December as shown in Figure 1. Annual precipitation was 2,210 mm. The mean precipitation recorded during the rainy (December through March) and dry seasons (June to October) was 2,124 mm and 86 mm, respectively. The total precipitation registered during the rainy season varied between 1,917 mm in 2016 and 3,063 mm in 2017, while the highest precipitations were recorded in March in both cases (Figure 2). Precipitations measured during the dry season were 75 mm in 2016 and 193 mm in 2017, respectively. The average monthly air temperature and relative humidity values measured during the study period were 24°C and 82%, respectively. Average, maximum, and minimum air temperature and relative humidity values were 27 and 22.6°C and 90% and 76%.

Trees of cocoa clone CCN 51 selected for the study were 18 years old and were planted in double rows, under full sunlight, with a spacing of  $2 \times 3$  m between trees and a distance of 4 m between double rows, which resulted in a density of 1,428 trees ha<sup>-1</sup>. The plantation was divided into eight lots of approximately 17-18 ha each. Regular irrigation was provided to the plots to ameliorate drought conditions. The trees were watered 2-3 times a week, depending on the season, and maintained 20–30% below soil water capacity. The irrigation system consisted of sprinklers that were placed following a distribution of  $14 \times 16$  m within the plots. The system delivered approximately 24 mm of water each week. However, this volume varied according to



FIGURE 1: Monthly precipitation registered during 2016 and 2017. Data are supplied by the Meteorological Station of Pichilingue (INAMHI) located in the province of Quevedo, Ecuador.

precipitation frequency and the season; during the dry months, season trees were consistently watered three times a week. During 2016 and 2017, the trees were fertilized once a year with 480 g/tree of a commercial preparation of NPK-Mg (19-6-26-3 + microelements). Weed control was carried out every 3 months. A first and more intensive pruning was conducted in December each year, to preserve crown architecture; later, a second, more moderate pruning was performed between March and April, to increase the amount of sunlight between the branches and reduce the incidence of pathogen-induced diseases. Regular pruning was also carried out, when needed, to eliminate unwanted branches.

2.2. Dynamics of Monthly Yield and Number of Pods by Size Categories. Pods were harvested in 8 lots during 2016 and 2017. The cocoa beans were later extracted and were predried in the sun for 8 hours to remove excess mucilage. Then, the fermentations were carried out in jute fiber bags for 5 days. Subsequently, the fermented cocoa beans were sun-dried (5-6 days) until a final moisture content of <8% was reached. In May 2017, 30 trees were randomly selected from three different lots and marked. The total number of pods in each tree selected was tallied weekly for six consecutive months and classified according to their length and diameter, into six separate categories, namely, Class 0, which contained cherelles with length  $\leq 3 \text{ cm}$ ; Class I, pods with a length between 4 and 12 cm; Class II, pods with lengths between 13 and 15 cm and diameters  $\leq$ 5 cm; Class III, pods with the length ranging between 16 and 18 cm and a diameter of 7 cm; Class IV, pods with lengths ranging between 19 and 22 cm and diameter  $\leq 11$  cm; and Class V, pods with lengths  $\geq 22$  cm and diameters  $\geq 11$ . The classes were defined according to the three stages of cocoa pod growth mentioned by Ten Hoopen et al. [17]: stage I cell division, stage II cell elongation, and stage III maturity. The cherelle stage has been defined as small pods less than 3 cm. Class I comprises the remaining stage of stage I. Classes II and III comprise the cell elongation stage, and classes IV and V include the stage where the fruit and the seed have reached physiological maturity and the ripening stage takes place. Ripe pods were always harvested, regardless of their size.

2.3. *Growth Curves.* A total of 4 cherelles were selected in August of 2017 in each lot (32 in total); these were marked and measured weekly to register weekly increments of length

and width for six consecutive months. Pod size increments were used to estimate growth curves according to the Richards function, which consists of an improved logistics equation that has been successfully used to estimate growth rates in cacao pods [17]. The Richards function states that

$$Y(t) = A + \frac{K - A}{\left(1 + Qe^{-Bt}\right)^{1/\nu}},$$
(1)

where A is the lower asymptote, K is the upper asymptote, B is the growth rate, t is the time interval, v > 0, and Q refers to the initial value Y (0). The growth parameters were obtained from a least-squares regression using the Marquardt–Levenberg algorithm. In addition, for both length and width, we utilized Google Colab in combination with the scipy.optimize module. We employed the "least\_squares" function to determine the values of A, K, Q, B, and v.

#### 3. Results and Discussion

To our knowledge, this is one of the first studies that deal with the dynamic distribution of cocoa pods concerning their size. The objective of this study was to understand the distribution of number of pods over time and the numbers of the different sizes of pods that can be found in the tree.

3.1. Pod Production and Yield Dynamics. The total annual yield recorded in clone CCN 51 during 2016 and 2017 was 1,809 and 1,993 kg ha<sup>-1</sup>, respectively, as shown in Figure 3. In both cases, the highest yield was recorded from October through December, which are the last three months of the annual dry season. During this period, monthly yield values remained above  $312 \text{ kg} \cdot \text{ha}^{-1}$ . November accounted for the month with the yield with an average of  $523 \text{ kg} \cdot \text{ha}^{-1}$ . In contrast, the period of March–July accounted for the one with the lowest yield of the year and coincided with the end of the annual rainy season and the transition toward the drier months, during which the mean monthly values dropped to 20–60 kg \cdot ha^{-1}.

According to García-Briones et al. [18], the highest yields of cacao reported nationwide in Ecuador occur between October and January, regardless of the amount of water supplied to the plantations. However, pod production rates may vary seasonally depending on the cultivar. Previous studies have allowed us to identify the clones that are more productive during the rainy season and those that are more



FIGURE 2: Monthly averages of the mean, maximum, and minimum of the air temperature and relative humidity, monthly averages of evapotranspiration, and precipitation from the period January 2016 to December 2017 (Data of Pichilingue station of Institute of Meteorology and Hydrology (INAMHI) at Quevedo Canton, Los Rios Province, Ecuador.

productive during the dry one [19]. The clone CCN 51 selected for this study outstands for its high productivity during the dry season in coastal regions of Ecuador, a trait that is most likely related to the intrinsic traits (self-compatible and cross-compatible clone, a high number of retained flowers compared to other clones) described by Jaimez et al. [13]. Nevertheless, we may infer that regular,

year-round irrigation enhances its productivity, when comparing fruit yield  $(1,800-1,900 \text{ kg ha}^{-1})$  against the mean yield values  $(1,050 \text{ kg ha}^{-1})$  reported for plantations of the same clone lacking irrigation, cultivated in other localities of Ecuador, such as, Quevedo and Tenguel [16]. According to Boza et al. [20], clones of CCN 51 have yielded 50 to 70% higher than the clone "Nacional" type, established in the



FIGURE 3: Cacao yield (kg  $ha^{-1}$ ) dynamics estimated in the clone CCN 51. Data were taken from 8 plots of 19 ha each, cultivated in the province of Quevedo during 2016 and 2017.

same region of Quevedo. The physiological mechanisms that regulate the flowering phenology of the clone CCN 51 are not known but appear to be synchronized with the end of the rainy season and the onset of the dry one, unlike the typical phenological patterns described in cacao, which generally synchronize the peak of flowering with the rainy season [21].

The higher fruit production observed in clone CCN 51 during the end of the dry season in Ecuador suggests that the highest rates of flower pollination are likely to occur between June and July, whereas pod growth occurs between June and September. It is also advisable to keep in mind that the dry season along the Ecuadorian coast is characterized by a low irradiance, in terms of photosynthetic photon flux density (PPFD), as well as a lower vapor pressure deficit (DPV). These conditions maintain high relative humidity (RH), which ameliorates environmental stress, reducing the evaporative demand. On the other hand, the decline in precipitations during the dry season also reduces the incidence of frosty pod rot disease, which causes severe losses in fruit production during the first stages of cacao pod development [17]. Although drier conditions may be beneficial for pod development and maturation, the drawback is that they limit the proliferation of cacao-pollinating insects that require elevated humidity for their reproduction [22], which can, in turn, lead to a decrease in the number of fruits originated from cross-pollination. However, both selfpollination and cross-pollination have been described in clone CCN 51 [23], ensuring its high productivity, thanks to its high self-compatibility index (number of fruits/number of pollinated flowers) according to Vera et al. [24]. According to previous studies, Clone CCN 51 has a pod index value of 15.2 [15, 25, 26].

3.2. Dynamics of Pod Size Distribution. The maximum mean values of the number of pods/tree (36) were recorded between mid-July and mid-August; these values corresponded to the period in which the broadest range of sizes was observed (Figure 4(a)). By September, the number of pods/ tree declined consistently, dropping to 5–7 pods/tree by December. During the period of the highest pod production, approximately 50% of the pods had lengths ranging between

3 and 12 cm and 17% were in the size classes associated with pods in the stage of cherelles (Figure 4(b)). The number of pods with longitudes between 13 and 18 cm never exceeded 9 (classes II and III) and were recorded only between July and December (Figure 4(c)). The number of pods with lengths ranging between 19 and 22 cm increased significantly by July and reached a maximum of 13 pods tree<sup>-1</sup> by mid-September. The following months were characterized by a marked decline in the production of pods, which lasted until December (1 pod tree<sup>-1</sup>) (Figure 4(d)). From July onwards, a small increase in the number of pods with lengths greater than 23 cm was recorded, which reached maximum size increments by the first two weeks of October; yet, the mean number of pods that exceeded this length was 7 pods/ tree. Afterward, the number of pods decreases due to the harvest (Figure 4(d)).

The mean monthly number of harvested pods/tree was  $3.4 \pm 0.3$  (September),  $2.4 \pm 1.7$  (October),  $9.3 \pm 0.4$  (November), and  $5.4 \pm 2.9$  (December) which represent the means of 90 trees  $\pm$  standard error. The mean total harvested fruits were 18 mature pods tree<sup>-1</sup>, which represented an annual of 25704 pods ha<sup>-1</sup> in 2017.

Based on these findings, the highest number of pods/tree recorded during the fructification season in clone CCN 51 corresponded to the stage in which approximately 65% of pods had lengths between 3 and 15 cm. Therefore, premature pod abscission is foreseeable, which occurs due to high competition between pods for the same resources. Premature pod abscission may be minimized by maintaining adequate fertilization and irrigation and proper timing of pruning practices since the latter can induce the growth of new branches that will increase the number of carbon sinks in the tree and compete with the developing pods for available photoassimilates on the same tree. The distribution of assimilates toward pods is directly related to the growth rate and the number of pods of each size class. For example, the lowest rate of pod growth reported in cacao corresponds to the cherelles and pods of classes IV and V, according to the growth curve obtained and to the physiological model developed for cacao by Zuidema et al. [27]. To date, the distribution paths of assimilates in the cacao tree remain uncertain [7], yet the distribution and concentration of photoassimilates will likely vary at any given moment, depending on the number and size of the fruits, as well as on the water content and nutritional status of the soil.

The stage that accounts for the highest number of pods with lengths equal to or superior to 19 cm (Class V) also coincides with the stage in which the number of cherelles decreases. It is also likely that during this phase, existing photoassimilates may be also directed toward leaf formation, as well as pod maturation, rather than toward the formation of flowers and new fruits. Based on the obtained results, the selective allocation seems to occur between the first week of September and lasts until the first week of October. These results suggest that the distribution of photoassimilates to the existing sinks is indeed a dynamic process within the same tree, during the entire season of fruit production and maturation.



FIGURE 4: Number of pods tallied in each size class established to describe pod growth in cacao trees of the clone CCN 51, planted in Quevedo, Ecuador. (a) Total number of pods per plant. (b) Number of cherelles with a length  $\leq$ 3 cm assigned to Class 0 (closed circles) and number of pods with lengths between 3 and 12 cm assigned to Class I (open circles). (c) Number of pods assigned to Class II (closed circles), defined by lengths of 13–15 cm and widths of 5 cm, and the number of pods assigned to Class III (open circles), defined by lengths of 7 cm. (d) Number of pods assigned to Class IV (closed circles), defined by lengths of 19–22 cm and widths of 11 cm, and the number of pods assigned to Class V (open circles), with lengths  $\geq$ 22 cm. Circles represent mean values based on measurements of pods harvested from 90 trees. Bars represent standard error values.

3.3. Pod Growth Rate. According to the obtained findings, the development time of the pods produced by the clone CCN 51 is 150 days, counting from the moment when the pods are formed until they reach full maturity and are ready to harvest. The highest pod length recorded in trees of these clones during this study was 24 cm, and the highest diameter was 10 cm. Growth increments appeared to stabilize after 100-105 days, and the following 45 days correspond to the stage of pod maturation (Figure 5). According to the growth curves estimated using the Richards function, the highest rates correspond to the linear phase of the curve, which represents the stage in which the daily increments of length and width reach 1.8 mm and 0.8 mm, respectively (Figure 6). We have employed the least-squares method once again, utilizing the Marquardt-Levenberg algorithm, applied to the data points calculated between day 7 and day 91, using the linear function.

The relatively low number of pods accounted for in classes II and III throughout the fruiting period has been attributed to their high length growth rates in both cases ( $0.19 \text{ cm} \cdot \text{day}^{-1}$ ). This daily increment suggests an increase of 5 cm approximately between classes over 20 days (from 13 cm in pods belonging to Class II to 18 cm in Class III). Growth rates, in terms of size increments, slow down once pods reach a length of 19-20 cm. This decline coincides with the onset of the ripening phase, in which the embryo consumes the seed's endosperm [17].

A period of 110 days has been estimated for the pods to complete their expansion and a period of maturation of 40–50 days to ripen. This timeframe is consistent with the one reported in previous studies for this clone, keeping in mind that the duration of the maturation process differs according to the criteria that each researcher employs to describe the entire growth cycle. Doaré et al. [28], for example, have established a fructification cycle of 160 days; counting from the moment pollination occurs until completing pod development in cacao trees cultivated in French Guiana. Studies conducted in the latter reported fructification cycles ranging from 132 to 170 days, depending on the cultivar. According to these authors, the relatively extended



FIGURE 5: Growth curves for both length (a) and width (b) in pods from their initial formation until reaching full maturity, ready for harvest, in the CCN 51 clone. These curves are modeled according to the Richards formula. Each data point represents the mean value based on observations from 12 pods. For the length, the parameter values are A = -227.64, K = 175.35, Q = 2,357,414,032.28, B = 0.23, and  $\nu = 39.82$ . For the width, the parameter values are A = -13.46, K = 82.72, Q = 416,024,324.23, B = 0.21, and  $\nu = 11.58$ .



FIGURE 6: Points obtained between days 7 and 91 using the Richards function for length (a) and width (b), respectively. In both cases, the least-squares method was employed on the data points with a linear equation. For length, the slope is 1.817 and the intercept is -0.935. For width, the slope is 0.777 and the intercept is -5.549.

cycle depicted in clone CCN 51 is associated with the production of larger, thus heavier cocoa beans. This logistic model, used by Muniz et al. [29] to describe cacao pod growth rates in a Brazilian cultivar, was characterized by a fructification cycle of 150 days, with rapid growth rates during the first 105 days of pod development, followed by a marked decline in the remaining 45 days. Variations in the number of days required to reach maximum growth rates are also influenced by environmental parameters, such as the mean air temperature [27, 30]. Recently, Goudsmit et al. [31] using Richards' function found that pod growth rates are related to the period of the year in which they are developing. In times of high production peaks, the growth rates are higher than in periods of lower production peaks. Growth rates in both seasons are greater if the plants are fertilized and the time at which maximum growth occurred 96 days after during the major and 86 days after during the minor season. Higher average temperatures can also reduce the number of days involved in fruit development and maturation. The elevation is another parameter that needs to be considered when estimating the duration of the fructification cycle. In the specific case of Ecuador, the clone CCN 51 is generally planted at elevations ranging from sea level up to 450 m; therefore, depending on the elevation and microclimate conditions, this cycle of 140-160 days is subject to variations.

It is important to keep in mind when estimating cultivar and/or clone productivity, there will always be discrepancies between the actual number of pods harvested and the total number of pods produced during the fructification period. This study supports this affirmation, given that only 55% of the total number of fruits produced by this clone completed their development and were harvested. The discrepancies between the number of pods produced and those that reach maturity could be attributed to the competition that arises between the pods when the concentrations of photoassimilates become limited, and to the losses originated by disease and/or insect attacks as well.

3.4. Fruit Production and Crop Management. The dynamics of the pods of different sizes indicate that the moment where the greatest number of cherelles is obtained is the period in which adequate fertilization must be provided to avoid limitations in growth, because classes II and III are when the pods are in the linear phase of growth (highest growth rates). Therefore, in each region, such a period should be known, and it is subject to climatic variability. This would help in fertilization plans. This period should be accompanied by an adequate supply of irrigation. However, this recommendation is a limitation for small producers who lack irrigation and plan fertilization based on the rainy and dry seasons.

It seems that there is a moment of low mobilization of carbohydrates to the pods, which corresponds to the moment of the lowest number of cherelles and pods in classes I, II, and III and coincides with the highest harvest peak. The end of this period would probably be an adequate period for pruning in the studied location which coincide with the beginning of the rainy season. However, additional studies are necessary to evaluate at least two years of dynamics and complement it with temperature data that, although they seem to vary little, the magnitude of the changes influences the time to reach maturity.

#### 4. Conclusions

The fruiting cycle of clone CCN 51 was characterized by the coexistence of fruits belonging to a wide range of growth states. However, the number of pods belonging to each particular size class differed throughout the fruiting season, allowing us to identify the times when the greatest number of pods of different size class occur. The information obtained revealed that the mean maximum number of pods produced by the widespread cocoa clone CCN 51 in conditions of full sunlight is 36 pods/tree, of which 65% will have a length ranging between 3 and 15 cm. Given that during this stage the pods reach their highest demand of photoassimilates during their development, it is essential to provide adequate fertilization and regular irrigation to diminish the incidence of premature pod abscission during the harvesting season. As the fructification season advances, the demand for photoassimilates of each size class changes, and the number of pods belonging to the classes with the highest growth rates (classes II and III) diminishes. The obtained results also confirm that the maximum rates of cocoa pod production in clones of CCN 51 cultivated in Northern Ecuador occur in November and coincide with the end of the six-month dry season that characterizes this region. The logistic model represents a valuable tool to determine pod growth rates in the clone CCN51-one of the most relevant clones in the cocoa industry. Nevertheless, future research is still required to evaluate the accuracy of the obtained model to evaluate pod growth in different locations.

#### **Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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