

Intercellular Space Volume Determination of Citrus Fruits Using Multiple Linear Regression

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Abstract—Internal concentration of CO₂ and O₂ is influenced by intercellular space volume (V_{in}). V_{in}, which located inside fruit, is thought to be a diffusion pathway through plant organs to supply adequate O₂ for respiration and allow diffusion of CO₂. This indicates that V_{in} is an important factor to be known. Unfortunately, measuring of V_{in} is time consuming. Therefore, an aim of present study is to determine intercellular space volume (V_{in}) of citrus fruits using multiple linear regression based on physical measurements such as mass (m), volume 1 as Archimedes principle (V₁), volume 2 as sphere (V_s), vertical dimension diameter (vd), horizontal dimension diameter 1 (hd1), horizontal dimension diameter 2 (hd2) and real density (P_r). Sample was physically measured to obtain its mass, diameters, volumes, and real density. The values of physical measurements were used to predict V_{in} by using four prediction models. Multiple regression was engaged to develop each category model. The model in equation (V_{in} = 0.234–0.932m+0.993V₁) had the highest score in determination coefficient (R²=0.998) and the lowest in root mean squared error (RMSE=1.533), which implied the best equation to determine V_{in}.

Index Terms—determination, prediction, model, intercellular space volume, citrus

I. INTRODUCTION

Japan is one of the major citrus producing countries in the world. One of the main regions in Japan that produce citrus is Ehime prefecture. As many as 20 major citrus varieties are being cultivated in Ehime throughout a year, such as Siranui, Iyo, ‘Kiyomi’ tangor, Ponkan, Unshu, and Navel orange.

The quality of fresh produce is greatly affected by internal concentrations of CO₂ and O₂ [1], which is influenced by the intercellular space volume (V_{in}). In this process, V_{in} acts as a diffusion pathway through plant organs to supply adequate O₂ for respiration [2] and allow diffusion of CO₂. Moreover, in the gaseous transport of O₂, CO₂, C₂H₄ and other physiologically important gases, V_{in} was also described as the network system for gaseous transport [3], [4].

There have been few studies of V_{in} in any of fruits and vegetables. Studies on gas exchange in fruit have shown that V_{in} inside the fruit is an important parameter to be investigated [1], [5]-[8]. Another study has demonstrated

that a fruit tissue with small V_{in} results low respiration [9]. Other studies focusing on V_{in} in apple and pear fruit have shown that fruits with greater V_{in} or porosity, that is the ratio of V_{in} and total volume of a fruit, had higher internal gas diffusion [10] and were softer [11] or more mealy [12]. Furthermore, study on the tolerance to Controlled Atmosphere (CA) conditions has also suggested that this tolerance depends on V_{in} or porosity. For instance, the cultivar (cv) Cox Orange is characterized by low porosity and has a low tolerance to CA, while fruits with high porosity tend to have high tolerance to CA [13]. From the above studies, it can be concluded that V_{in} is also an important factor in estimating sensitivity to low levels of O₂ and/or high CO₂ respiration and in determining appropriate concentrations for CA storage [13].

Some techniques have been developed to measure V_{in} in fruit. One is based on gasometric method that measured the amount of air extracted from sample when subjected to vacuum [14]. Next, method based on experiment of Archimedes principle V_{in} is measured as a difference between the impelling force acting on an organ with and without V_{in} [4]. Another method is pycnometric method where V_{in} is estimated as a difference between the fruit volume measured by water immersion minus its volume without V_{in}, estimated after maceration with a pycnometer technique [15].

In this research, pycnometric method that follows the Archimedes principle was used in order to measure V_{in} because this method is appropriate for organ with internal with internal cavities and succulent tissues [3], for example citrus. Furthermore, this method seems to be accurate for measuring V_{in} [4]. Unfortunately, this technique is much more time consuming and the destruction of the samples is the major limitation of this technique [4], [15]. To solve this problem, determination of V_{in} by prediction is a necessary. However, there were no detailed studies concerning V_{in} prediction of citrus in the literature. Therefore, this study was carried out to develop appropriate models to predict V_{in} by multiple linear regression based on physical measurements (mass, volume, diameters, and real density).

II. MATERIAL AND METHOD

A. Materials

The experiment employed eight different species of freshly harvested citrus at Ehime University farm, Iyo

(*Citrus iyo Hort. ex Tanaka*), Navel (*Citrus sinensis L. Osbeck var. brasiliensis Tanaka*), Ponkan (*Citrus reticulata Blanco*), Siranui (*Citrus unshiu Marcov x Citrus sinensis Osbeck x Citrus reticulata Blanco*), Amanatsu (*Citrus natsudaidai Hayata*), 'Kiyomi' tangor (*Citrus unshiu Marcov. forma miyagawa-wase x C. sinensis Osbeck*), Lime (*Citrus aurantifolia*) and Unshu (*Citrus unshu Marcovitch forma Miyanaga-wase*). Every species consisted of two sizes (L and 2L), except Lime, for which were used (small and large) due to the absence of size standards. Citrus fruits were measured just after harvest (Table I).

TABLE I. HARVEST DATE AND SAMPLE SIZE PROPERTIES OF EIGHT DIFFERENT CITRUS SPECIES INCLUDING THEIR DIAMETERS, VERTICAL DIMENSION (VD) AND HORIZONTAL DIMENSION (HD)

Harvest date	Cultivar	Size	Diameters (cm)	
			vd	hd
22 January	Navel	L	7.6	7.7
		2L	8.0	8.2
23 January	Iyo	L	7.5	8.5
		2L	7.8	9.2
8 February	Ponkan	L	6.2	6.7
		2L	6.5	7.5
4 March	Siranui	L	7.2	7.7
		2L	7.6	8.3
13 April	Amanatsu	L	7.6	9.7
		2L	8.1	10.2
3 March	'Kiyomi' tangor	L	6.1	6.8
		2L	6.7	7.8
5 November	Lime	Small	5.0	4.8
		Large	6.2	5.6
4 December	Unshu	L	5.1	7.1
		2L	5.4	7.7

Note: All measurements were performed in quintuplicate (n=5) in 2013. HD was obtained from mean values of hd1 and hd2 perpendicularly intersecting each other.

B. Physical Measurements

The measurements of mass, diameter, real density and volume were performed for 5 whole fruits for each size respectively so that 10 fruits were measured for every species.

1) Mass and diameters

Fruit mass (m) was measured using an electric balance accurate to 0.1g. Vertical dimension (vd), horizontal dimension 1 (hd1), and 2 (hd2) which are perpendicularly intersecting each other were measured by a slide calipers accurate to 1 mm. Vertical dimension represented the measurement from one apex to the next. Horizontal dimension represented the measurement of equatorial axis (Fig. 1).

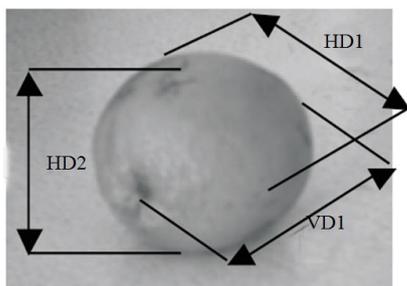


Figure 1. Three diameters dimension of citrus

2) Volume

A technique based on Archimedes' principle was used in order to determine the total volume (V_t) of fruit. Buoyancy was measured using an electric balance accurate to 0.1g when a sample was submerged in water. Sphere equivalent volume (V_s) was also calculated by assuming that the fruit is spherical.

3) Real density

Real density (P_t) of citrus tissue was measured by the pycnometric technique [15]. First, a pycnometer fully filled with distilled water before was weighed (W_1) by an electric balance accurate to 0.001g. The density of distilled water was 0.9982071g/cm^3 while the temperature of the distilled water was kept on 20°C . Next, the water in the pycnometer was removed. After that, pycnometer was weighed (W_2). Approximately 10g of ground sample that is one-eighth of a whole sample (stem end, consisting of peel and flesh mixture) was placed in the pycnometer and weighed using the electric balance (W_3). A sample weight in the pycnometer can be obtained by subtracting W_3 to W_2 (sample weight = $W_3 - W_2$). Next, sample in the pycnometer was added of some water; subsequently it was vacuumed with 0.01MPa for up to 30 minutes to eliminate the air bubbles contained in the sample. Next, the pycnometer containing the sample was then filled fully with distilled water and weighed (W_4). Finally, real density (P_t) was calculated by using (1).

$$P_t = \frac{\text{sample weight}}{W_1 + \text{sample weight} - W_4} \quad (1)$$

0.9982071

4) Determination of intercellular space volume and porosity

Intercellular space volume (V_{in}) was calculated after measuring real density and total volume. V_{in} is the difference between total volume and volume of fruit tissue (V_{tissue}) [15]. Therefore, V_{in} was calculated by using (2) or (3).

$$V_{in} = V_t - V_{\text{tissue}} \quad (2)$$

$$V_{in} = V_t - \frac{m}{P_t} \quad (3)$$

V_{in} : Intercellular space volume (cm^3)

V_t : Total volume of whole fruit (cm^3)

V_{tissue} : Volume of fruit tissue (cm^3)

m: Mass of fruit (g)

P_t : Real density of fruit tissue (g/cm^3)

Porosity (\emptyset) was determined using the following equation:

$$\emptyset = \frac{V_{in}}{V_t} \times 100 \quad (4)$$

\emptyset : Porosity (%)

5) Measurement of moisture content

Moisture content was measured by the oven drying method (100°C for 24 hours). Sample of about one-eighth of a whole fruit was used for the measurement. All samples were weighed using an electric balance accurate to 0.001g. Wet basis moisture content results was presented as percentage (%).

6) Statistical analysis

Real density values were analyzed by one-way analysis of variance (ANOVA) based on cultivar while the significance of differences among means of cultivar were determined using Tukey's test. The level of significance was set as $P < 0.05$. SPSS software for windows version 16.0 (SPSS Inc., IL, USA) and Microsoft Excel 2010 were employed to analyze the data.

III. RESULTS AND DISCUSSION

A. Physical Measurements

The physical measurements such as mass, volume, and real density of eight different cultivars of citrus are shown in Table II.

Table II shows two physical measurements values of seven citrus cultivars with L and 2L sizes and one citrus cultivar (Lime) with small and large sizes. It is clear that in term of mass, and volume, lime cultivars in both sizes had the lowest mean values among the citrus cultivars. For instance, the physical measurement for mass (61.2g), volume of V_t , V_s (62.0cm³, 59.6cm³) in L size, and accordingly mass (101.6g), and volume of V_t , V_s (104.0cm³, 100.7cm³) for 2L size. Amanatsu cultivar, meanwhile, had the highest means value in both sizes of cultivars. It had mass (342.5g), and volume V_t (404.7cm³), V_s (370.8cm³) for L size, and mass (391.3g), V_t (473.2cm³), and V_s (445.9cm³) for 2L size. It is followed by Iyo cultivar in the second and Navel cultivar in the third ranks.

TABLE II. PHYSICAL MEASUREMENTS OF EIGHT DIFFERENT CITRUS CULTIVARS

Cultivar	Physical measurements	Size of citrus*				
		L (n=5)		2L (n=5)		
Navel	Mass (g)	224.2	± 9.2	272.4	± 10.5	
	volume (cm ³)	V_t	243.8	± 11.4	297.7	± 15.3
		V_s	236.5	± 15.4	284.9	± 10.5
Iyo	Mass (g)	248.3	± 13.9	290.3	± 4.6	
	volume (cm ³)	V_t	306.5	± 15.0	377.9	± 16.7
		V_s	284.0	± 13.6	346.0	± 23.5
Ponkan	Mass (g)	125.6	± 11.4	162.1	± 15.2	
	volume (cm ³)	V_t	155.4	± 10.9	205.8	± 9.1
		V_s	146.0	± 7.8	192.9	± 8.4
Siranui	Mass (g)	199.1	± 10.0	241.4	± 15.6	
	volume (cm ³)	V_t	226.8	± 10.0	269.1	± 16.1
		V_s	225.2	± 7.7	270.6	± 21.1
Amanatsu	Mass (g)	342.5	± 16.9	391.3	± 23.6	
	volume (cm ³)	V_t	404.7	± 28.0	473.2	± 27.1
		V_s	370.8	± 35.2	445.9	± 31.1
'Kiyomi' tangor	Mass (g)	135.7	± 7.9	199.8	± 19.2	
	volume (cm ³)	V_t	153.6	± 3.9	224.3	± 21.5
		V_s	148.1	± 11.4	218.8	± 22.7
Lime	Mass (g)	61.2	± 5.8	101.6	± 4.7	
	volume (cm ³)	V_t	62.0	± 5.9	104.0	± 4.1
		V_s	59.6	± 3.6	100.7	± 2.6
Unshu	Mass (g)	137.4	± 3.7	168.8	± 6.4	
	volume (cm ³)	V_t	152.6	± 3.8	191.9	± 13.5
		V_s	134.6	± 4.2	166.6	± 9.8

Note: All measurements were performed in quintuplicate (n=5). Data are expressed as the mean ± SD.

*All cultivars were classified into two sizes L and 2L, except Lime was Small and Large

For real density among species, Unshu and Lime were grouped together and the six remaining species were grouped together. To investigate the differences in real density among species, the relationship between moisture content and real density was investigated. As shown in Table III, species with greater moisture content had less real density, and vice versa. For instance, while the moisture content and real density of Lime and Unshu are about 86.29% and 1.0531g/cm³, respectively, the corresponding values for Navel, Iyo, Ponkan, Siranui, Amanatsu, and 'Kiyomi' tangor are about 82.96% and 1.0678g/cm³. This result was consistent with a study by Abhayawick *et al.* (2009), in which the moisture content of three onion varieties was investigated (Sweet Vidalia, Spirit and Niz); the onion varieties with higher moisture had lower real density.

TABLE III. REAL DENSITY AND MOISTURE CONTENT OF SIZE L AND 2L FOR EIGHT CULTIVARS OF CITRUS FRUITS

Cultivars	Real Density* (g/cm ³)	Moisture content** (%)
Navel	1.0676 ^a	81.32
Iyo	1.0655 ^a	84.53
Ponkan	1.0700 ^a	83.22
Siranui	1.0688 ^a	81.02
Amanatsu	1.0651 ^a	83.24
'Kiyomi' tangor	1.0697 ^a	84.41
Lime	1.0562 ^b	86.53
Unshu	1.0500 ^b	86.05

Note: *All measurements were performed in quintuplicate (n=5) for every species.

**All measurements were performed in solo (n=1).

Means of real density followed by different letters are significantly different as determined by the Tukey test at $P < 0.05$.

Interestingly, all of the cultivars mostly had similar real density value. A total mean values was 1.0640g/cm³, ranging from minimum 1.0409g/cm³ to maximum 1.0838g/cm³ with standard deviation 0.0091g/cm³ and standard error 0.0010g/cm³ (data have not shown). In general, the result was similar to previous study stating that real density of Mandarin citrus was around 0.81 to 1.08g/cm³ [16]. Then, from these low standards (deviation and error), it can be concluded that value of real density to all cultivars is around 1.0640g/cm³, even though it may result a significant different according to statistical test (Table III).

B. Intercellular Space Volume and Porosity

Table IV compares the values of intercellular space volume (V_{in}) and percent porosity of eight different citrus cultivars with two sizes. Iyo, Ponkan and Amanatsu have average of porosity about 24.2%. This finding has almost similar results with previous study where Mandarin citrus had porosity about 24% [17].

TABLE IV. REAL DENSITY AND MOISTURE CONTENT OF SIZE L AND 2L FOR EIGHT CULTIVARS OF CITRUS FRUITS

Cultivar	Size	Intercellular space volume (cm ³)		Porosity (%)	
Navel	L	33.5	± 4.9	13.7	± 1.5
	2L	42.7	± 7.7	14.3	± 2.0
Iyo	L	73.5	± 13.0	23.9	± 3.7
	2L	105.2	± 12.0	27.8	± 1.9
Ponkan	L	38.0	± 7.9	24.5	± 4.8
	2L	54.3	± 12.8	26.4	± 6.2
Siranui	L	40.9	± 8.2	18.0	± 3.3
	2L	42.8	± 7.0	15.9	± 2.2
Amanatsu	L	83.8	± 12.2	20.6	± 1.7
	2L	104.9	± 10.6	22.2	± 2.0
'Kiyomi' Tangor	L	27.9	± 10.4	17.8	± 5.1
	2L	36.8	± 4.7	16.4	± 1.5
Lime	Small	4.2	± 0.8	6.7	± 0.8
	Large	7.6	± 0.4	7.3	± 0.6
Unshu	L	21.9	± 2.5	14.4	± 1.5
	2L	30.8	± 8.0	15.9	± 3.1

Note: All measurements were performed in quintuplicate (n=5). Data are expressed as the mean ± SD.

The study has also displayed that V_{in} of each species is ranging from 4.2 to 105.2cm³, in which Iyo with 2L size had the higher V_{in} than other species. The difference of V_{in} in each species that can be a property of the cultivar as well as be a function on the growing season and the number and the size of cells is likely to explain whether Iyo species has higher V_{in} than others [18]-[20].

C. Determination of Citrus's Intercellular Space Volume by Multiple Linear Regression

The following two steps were applied in order to develop model of predicting citrus' V_{in} from measured dimension (mass, volume and real density). First, all categories models were developed by multiple linear regressions. This analysis is useful for acquiring a linear equation allowing the estimation of independent variable or criterion e when the value of the q independent or predictive variables x_1, \dots, x_q are known:

$$e = b_0 + b_1x_1 + \dots + b_qx_q$$

where the parameters b_0, b_1, \dots, b_q represent the contributions of each independent variable to the estimation of the dependent variable. Second, validation models by comparing actual value of V_{in} and prediction value from eight different cultivars.

Selection of the best model was based on which had a higher determination coefficient (R^2) value and a lower Root Mean Squared Error (RMSE) for all species. Both R^2 and RMSE are applicable to quantify the predictive ability during the validation phase and to identify the best model for predictions. First, the determination coefficient (R^2) describes the proportion of total variance in the observed data that can be explained by the model. Second, the Root Mean Squared Error (RMSE) is appropriate to quantify the error in the same units of the variables. The absolute error measures were used to calculate the square root of the mean squared error given by (5).

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (Q_t - Q_1)^2}{N}} \quad (5)$$

where Q_t is the observed value, Q_1 is the estimated value and N is the total number of observations of the validation set. To obtain an acceptable goodness of fit, the values of R^2 must approach one, while the values of RMSE must approach zero [21].

1) First category models, mass and volume

In the first category, regression models of V_{in} with mass and volume dimensions were divided into two categories. The first regression was based on the volume (V_t) of sample calculated by using the principle of Archimedes experiment, and the second regression was based on the volume (V_s) of sample that was assumed as a sphere. Both models derived through multiple linear regressions, where independent variables were dimension of mass (m) and volume (V_t, V_s) and dependent variable was (V_{in}).

Prediction of citrus V_{in} on the basis m and V_t was presented in (6) with 0.998 of R^2 and 1.543 of RMSE.

$$V_{in} = -0.358 - 0.940m + 1.002V_t \quad (6)$$

In term of determining the model to predict V_{in} based on m and V_s , multiple linear regression procedure was applied as same as in (6). As a result, (7) was recommended for predicting V_{in} with 0.891 of R^2 and 10.241 of RMSE.

$$V_{in} = -4.676 - 0.651m + 0.820V_s \quad (7)$$

Equation (6) and (7) were validated in every cultivar. See Table V for the result of the calculation of R^2 each equation. Every cultivar consists of 10 samples, where R^2 was calculated from each equation by comparing between actual measurement of the sample and predicted values of V_{in} . The R^2 values can be interpreted as the proportion of the variance in the prediction estimates attributable to the variance in the actual measurements [21]. In the other word, the model that has higher R^2 is better than lower R^2 .

Results indicated that in (6) as having greater value than did in (7). Almost all estimations in (6) had R^2 more than 0.900, meaning that this equation appropriate for predicting. Meanwhile, the result in (7) was low might

due to the sample had no precise sphere form. For example, Siranui that had distinctive form with large protruding bump on the top of the fruit might cause it had low of R^2 .

TABLE V. COMPARISON OF R^2 BETWEEN (6) AND (7) IN EIGHT DIFFERENT CULTIVARS OF CITRUS

Cultivars	R^2	
	equation (6)	equation (7)
Navel	0.939	0.639
Iyo	0.997	0.760
Ponkan	0.998	0.883
Siranui	0.953	0.351
Amanatsu	0.990	0.907
'Kiyomi' tangor	0.976	0.660
Lime	0.983	0.821
Unshu	0.989	0.628

Note: Equations were tested into every cultivar that consisted of ten samples

Indeed, the calculation in (7) was simpler than in (6). The result of (7) was simply from measuring the diameter of sample, while (6) was from experiment by using Archimedes principle. However, the result was inappropriate for predicting. The only sample that had R^2 more than 0.900 was Amanatsu having $R^2=0.907$.

2) Second category models, mass, volume, real density

In the second category, the model was found to be more responsive to estimate V_{in} of all cultivars based upon three physical measurements (mass, volume, real density). Taking into account all independent variables, the outcome of multiple linear regression method for the V_{in} of all citrus cultivars with R^2 coefficient of 1 and RMSE of 0.520 was found in (8).

$$V_{in} = -172.592 - 0.932m + 0.993V_t + 162.430P_t \quad (8)$$

However, (8) is not necessary to use it because by using (3), V_{in} can be calculated perfectly. Moreover, real density as an independent variable is difficult to be measured because of time consuming. Therefore, (8) can be simplified by substituting the value of P_t (1.0640) because according to our findings (Table III), this value is mostly similar to all cultivars. As a result, (9) was proposed to estimate V_{in} with $R^2=0.998$ and RMSE=1.533.

$$V_{in} = 0.234 - 0.932m + 0.993V_t \quad (9)$$

Similar to the first category model, this equation also replaced volume V_t with V_s . In other words, citrus was assumed as a sphere. The result is as presented in the (10) with $R^2=0.893$ and RMSE=10.228.

$$V_{in} = 155.288 - 0.681m + 0.849V_s - 150.655P_t \quad (10)$$

The (10) can be simplified by substituting the real density value of 1.0640 to this equation. The result is as presented in the (11) with $R^2=0.890$ and RMSE=10.179.

$$V_{in} = -5.009 - 0.681m + 0.849V_s \quad (11)$$

To validate the models approach, (9) and (11) were tested to every cultivar. The measured value (actual data) of V_{in} and the predicted value compared by using R^2 coefficient. A total of ten cases were used for validating

the models. The results of comparison between predicted and measured (actual) value of V_{in} to all cultivars citrus are shown in Table VI.

TABLE VI. COMPARISON OF R^2 BETWEEN (9) AND (11) IN EIGHT DIFFERENT CULTIVARS OF CITRUS

Cultivars	R^2	
	equation (9)	equation (11)
Navel	0.939	0.629
Iyo	0.997	0.757
Ponkan	0.998	0.887
Siranui	0.953	0.358
Amanatsu	0.990	0.905
Kiyomi Tangor	0.992	0.650
Lime	0.983	0.812
Unshu	0.989	0.620

Note: Equations were tested into every cultivar that consisted of ten samples

It is clear that, according to the Table VI, the result in (9) was greater than in (11). All computations in (9) had R^2 more than 0.900. It denotes that this equation appropriate for predicting V_{in} . In contrast, computation in (11) resulted low in R^2 value in all samples, except for Amanatsu.

From four model that have been presented it can be seen that the model in (9) ($V_{in} = 0.234 - 0.932m + 0.993V_t$) had the highest score in determination coefficient ($R^2=0.998$) and the lowest in root mean squared error (RMSE=1.533), which implied the best equation to predict V_{in} .

IV. CONCLUSION

In this paper, we have presented the determination of V_{in} in various citrus fruits and the development of a simple prediction method to estimate V_{in} . The values of both intercellular space volume (V_{in}) and porosity (\emptyset) vary among species. Another interesting finding is that the real density of all species was almost similar to the mean value, 1.0640 g/cm³. Because of this similarity, simple prediction methods to predict V_{in} were developed.

In this investigation, a model equation for determining intercellular space volume (V_{in}) of citrus has been developed which gives accurate predictions based on physical measurements. Although the model developed is mathematically simple, it is able to provide reliable determination of the intercellular space volume (V_{in}) of citrus. Experiments were conducted using eight different cultivar of citrus sample for modeling and evaluating the results predicted by the model. The results of model agreed closely with the experimental values. The mathematical formulations and the related assumptions which recommended to determine of V_{in} is $V_{in} = 0.234 - 0.932m + 0.993V_t$.

ACKNOWLEDGEMENT

The author thank to the Directorate General of High Education (DGHE) of INDONESIA for providing scholarship. This work was supported by a grant from JSPS KAKENHI Grant Number 24380135.

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