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Sampling plan for *Bemisia tabaci* (Hemiptera: Aleyrodidae) in melon crops

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Abstract

Melon (*Cucumis melo* L.; Cucurbitaceae) is one of the 10 most-consumed fruits in the world. The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important pests in melon crops worldwide. Conventional sampling plans are the starting point to establish decision-making systems for integrated pest management (IPM) programs. The purpose of this study was to determine a conventional sampling plan for *B. tabaci* in melon crops with plants at the vegetative, flowering, and fruiting stages. The best sampling units for *B. tabaci* were the 5th and 6th most apical leaves of the plant vine. The best sampling technique was direct counting of adult whiteflies. The most appropriate frequency distribution to describe *B. tabaci* densities in melon fields was the negative binomial. Whiteflies on melon fields with plants at different phenological stages showed a common aggregation parameter (*K*common = 0.9134). The optimal number of samples from the sampling plan was 72 samples per field with a maximum error of 15% in population determination. The sampling plan determined by this study can be used by farmers because it is a low-cost (US$5.27 per sampling), fast (39 minutes per sampling) and feasible (15% maximum evaluation error). The same sampling plan can be used with melon plants at the vegetative, flowering, and fruiting stages.

Key Words: *Cucumis melo* L.; sampling unit; sampling technique; negative binomial; decision-making

Resumen

El melón (*Cucumis melo* L.; Cucurbitaceae) es una de las 10 frutas más consumidas en el mundo. La mosca blanca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) es una de las plagas más importantes en los cultivos de melón en todo el mundo. Los planes de muestreo convencionales son el punto de partida para establecer sistemas de toma de decisiones para los programas de manejo integrado de plagas (MIP). El propósito de este estudio fue determinar un plan de muestreo convencional para *B. tabaci* en cultivos de melón con plantas durante de las etapas vegetativa, de floración y de fructificación. Las mejores unidades de muestreo para *B. tabaci* fueron la quinta y la sexta hojas más apicales de la planta enredadera. La mejor técnica de muestreo fue el conteo directo de adultos. La distribución de frecuencia más adecuada para describir las densidades de *B. tabaci* en los campos de melón fue el binomio negativo. Las moscas blancas en los campos de melón con plantas en diferentes estados fenológicos mostraron un parámetro de agregación común (*K*common = 0.9134). El número óptimo de muestras del plan de muestreo fue de 72 muestras por campo con un error máximo del 15% en la determinación de la población. El plan de muestreo determinado por este estudio puede ser utilizado por los agricultores por su bajo costo (US$5.27 por muestreo), rápido (39 minutos por muestreo) y factible (15% de error de evaluación máximo). El mismo plan de muestreo se puede usar con plantas de melón en las etapas vegetativa, de floración y de fructificación.

Palabras Clave: *Cucumis melo* L.; unidad de muestreo; técnica de muestreo; binomio negativo; toma de decisiones
Macêdo et al.: Sampling plan for Bemisia tabaci in melon crops

used to determine economic damage levels and to validate sequential sampling plans (Binns et al. 2000; Gusmão et al. 2006; Pedigo & Rice 2014; Rosado et al. 2014; Pereira et al. 2017). In conventional sampling plans, the number of samples is fixed, whereas in sequential plans this number is variable (Pereira et al. 2017; Pinto et al. 2017). Conventional sampling plans consist of a sample unit, sampling technique, and the number of samples (Gusmão et al. 2005; Moura et al. 2007; Bacci et al. 2008). Optimal sampling units and techniques must be accurate, fast, low-cost, and representative. Accurate sampling units and techniques have low variability. Thus, for units and techniques to be precise they must have a relative variance (RV) of less than 25% (Southwood 1978). On the other hand, representative units and sampling techniques are those that have relative densities (individuals per sample) that present the same variation as the absolute density (individuals per plant or vine). The number of samples in the sampling plans should be economically feasible. Therefore, sampling plans must be simple, fast, and cost effective in order to be viable for farmers (Gusmão et al. 2005; Moura et al. 2007; Bacci et al. 2008; Pinto et al. 2017).

Despite the importance of B. tabaci as a pest in melon crops, a sampling plan for use in IPM programs for this species has yet to be developed for melons. Therefore, the objective of this study was to determine a conventional sampling plan for B. tabaci in melon crops with plants during vegetative, flowering, and fruiting stages.

Materials and Methods

EXPERIMENTAL CONDITIONS

This study was carried out during 2015 and 2016 in 12 different commercial melon fields (variety 'Valenciano') in Formoso do Araguaia, Tocantins State, Brazil (11.9021055°S, 49.5616027°W, with an altitude of 240 masl, and a tropical climate with a dry winter). Each field consisted of approximately 20 ha. The plants were spaced by 1.5 × 2.0 m and established according to Braga Sobrinho et al. (2008) and Pessarakli (2016). The cultural treatments used were plant and fruit thinning, weed control, and fruit turning.

The research was divided into 3 parts. In the first part, the optimal sampling unit to assess B. tabaci was selected using the direct counting technique. In the second part, the optimal sampling technique (direct counting or leaf beating on a white tray) was selected. In the third part, the appropriate number of samples for the sampling plan was determined.

SELECTION OF THE OPTIMAL SAMPLING UNIT TO ASSES BEMISIA TABACI

This part of the study was carried out in 3 melon fields at different stages in 2015. The plants were evaluated in the vegetative, flowering, and fruiting stages (Figs. 1A, B, C). In each field, 100 plants were selected at random and in each plant, the largest vine was evaluated. The leaves were numbered according to their position on the branch. Leaf number 1 was the most apical of the vine, number 2 the 2nd most apical leaf, and so on (Fig. 1D). On each leaf, the number of B. tabaci adults was evaluated by direct count. In this evaluation, the leaf was handled carefully (to avoid insect escape) and the number of B. tabaci adults present was counted (Fig. 1E). The adults were evaluated because this is the insect stage that causes most damage to plants (Gusmão et al. 2006) and they are easier to count due to their larger size (Chu et al. 2003).

The sampling unit was selected based on the criteria of leaf occurrence frequency in a vine, precision, and representativeness. In the first criterion, only leaves with an occurrence frequency (frequency with which a leaf in a certain position appears in a vine) above 80% were selected. This was done due to the long time taken to locate leaves that have a low occurrence frequency on the vines, which can increase sampling time (Rosado et al. 2014; Pinto et al. 2017). The occurrence frequency of each leaf on the vines of melon plants was calculated using formula (1):

$$ Fi = \frac{(100 \times Ni)}{Nr} $$

where $Fi$ = frequency of occurrence of leaf $i$ (%) in the 100 vines evaluated, $i$ = position of the leaf on the vine (left to right), $Ni$ = number of times that the leaf $i$ was present on the 100 evaluated vines, and $Nr$ = total number of evaluated vines.

The precision criterion used to identify the sampling unit was the relative variances of B. tabaci densities on each leaf of the vine. Only relative variances of less than 25% were selected because samples and techniques with relative variance higher than 25% generate unfeasible sampling plans (Southwood 1978). The relative variances of B. tabaci densities on each leaf of the vine were calculated using formula (2) (Moura et al. 2003; Bacci et al. 2006):

$$ RV = \frac{(100 \times S(x))}{x} $$

where $RV$ = relative variance (%), $S(x)$ = standard error of the mean densities, and $x$ = mean densities.

Under the representativeness criterion, the relative densities (adults per leaf) and the absolute densities (adults per vine) were calculated, and the sample units considered were those with relative densities that were significantly correlated ($P < 0.05$) with absolute densities (Rosado et al. 2014; Pinto et al. 2017). The final selection was made based on the sample unit being amongst those suitable for the evaluation of pests according to all criteria during the vegetative, flowering, and fruiting stages.

SELECTION OF THE OPTIMAL SAMPLING TECHNIQUE

This part of the study was performed in 3 melon fields in 2015. During the evaluations, the plants of each field were in the vegetative, flowering, or fruiting stages (Figs. 1A, B, C). Twenty plants were randomly selected from each field. Two vines were evaluated in each plant. The evaluated leaves were those previously selected (fifth and sixth leaves). With 1 of these vines, B. tabaci density was evaluated by the direct count technique (Fig. 1E). With the other vine of the plant, whitely densities were evaluated by leaf beating onto a white tray (40 × 25 × 3 cm) and counting B. tabaci adults (Fig. 1F). These techniques were used because they are suitable for the evaluation of sucking insects such as B. tabaci (Moura et al. 2007; Pinto et al. 2017). The time required to execute each sampling technique also was evaluated.

The relative variance of B. tabaci densities obtained by each sampling technique was calculated. The calculation of relative variance was performed using formula (2). The data for B. tabaci densities and sampling times for each sampling technique were analyzed by analysis of variance at $P < 0.05$. The technique that provided a relative variance of less than 25% and a shorter sampling time was selected (Southwood 1978).

DETERMINATION OF THE NUMBER OF SAMPLES FROM THE SAMPLING PLAN

This stage of the study was divided into 2 parts. In the first part, the most appropriate frequency distribution was determined to describe the B. tabaci densities in the melon fields. In the second part, the maximum error to be used in pest sampling was determined.
Fig. 1. Crops with melon plants in phases (A) vegetative, (B) flowering, and (C) fruiting. (D) Leaves used as sample units (number 1 represents the most apical leaf of the vine and the largest number the most basal leaf of the vine). Sampling techniques of (E) direct count and (F) leaf beating on a white tray.
DETERMINATION OF THE FREQUENCY DISTRIBUTION THAT BEST DESCRIBES THE DENSITIES OF *BEMISIA TABACI* IN CROPS

This stage of the research was carried out in 12 melon fields during 2015 and 2016. During the evaluations, the plants of each field were in the vegetative, flowering, or fruiting stages (4 fields at each phenological stage). In each field, 300 plants were evaluated. These plants were located equidistantly to eliminate directional errors (Bacca et al. 2006; Rosado et al. 2014; Pereira et al. 2017; Pinto et al. 2017). The sampling techniques used were those previously selected (direct counting of *B. tabaci* adults on the fifth or sixth most apical leaves of the vine).

The observed and expected frequencies of *B. tabaci* densities were calculated according to the negative binomial, Poisson, and positive binomial distributions through Microsoft Excel spreadsheets. These frequencies were compared using the chi-square test ($\chi^2$) (Young & Young 1998). The data for the *B. tabaci* densities in a field were adjusted to a certain frequency distribution when the difference between the observed and expected frequencies was not significant (P > 0.05) (Young & Young 1998). A given frequency distribution was considered descriptive of *B. tabaci* densities in crops when the whitely distribution conformed to this frequency distribution in most fields (more than 70%) (Rosado et al. 2014; Pinto et al. 2017).

The values of the aggregation parameter for each field were calculated using formula (3) (Young & Young 1998; Moura et al. 2003):

$$k = \frac{x^2}{S^2 - x}$$

where $k$ = aggregation parameter in the field, $x$ = average density of the pest in the field, and $S^2$ = variance of the pest densities.

The aggregation parameters ($k$) of each of the 12 fields were assessed with simple linear regression analysis according to Bliss and Owen (1958). In this regression, it is considered that the fields present a common aggregation parameter ($kc$) when their intercept is not significant and the slope is significant by the F test at $P < 0.05$ (Rosado et al. 2014; Pinto et al. 2017).

**Determination of the Maximum Allowed Error for Calculating the Number of Samples**

Initially, the sample numbers of the sampling plan for melon crops in the vegetative, flowering, and fruiting stages were calculated using formula (4) (Young & Young 1998; Gusmão et al. 2005; Moura et al. 2007):

$$NA = \frac{1}{C^2} \times \left( \frac{1}{x} + \frac{1}{kc} \right)$$

where $NA$ = number of sample units, $C$ = permitted error, $x$ = population mean, and $kc$ = common parameter of aggregation of the negative binomial frequency distribution (0.9134) determined previously.

In these determinations, we used error values of 0.05, 0.10, 0.15, 0.20, and 0.25. These errors were used because they are the standard range of errors used in these calculations (Moura et al. 2007; Pinto et al. 2017). Given the number of samples in these situations, the sampling time for each of these plans was evaluated in 3 fields. The plants of each field were in the vegetative, flowering, or fruiting stages.

The sampling cost was calculated using the following formula (5):

$$CS = CM + (TS \times CL)$$

where $CS$ = cost of 1 sampling, $CM$ = cost of sampling material (pencil, rubber, paper, and clipboard: US$0.07 per sample), $TS$ = sampling time (h), and $CL$ = labor costs (salary of a rural worker and non-wage labor costs: US$8.00 per h) (Moura et al. 2007; Rosado et al. 2014).

**RESULTS**

**SELECTION OF THE PLANT COMPONENTS TO BE USED FOR *BEMISIA TABACI* SAMPLING**

In plants during the vegetative stage, only the 7 most apical leaves displayed frequencies of occurrence on the vines higher than 80%. From those 7 leaves, only the fifth and sixth leaves had whitefly density relative variances lower than 25%. From the third to seventh leaves, the relative densities (adults per leaf$^{-1}$) presented significant correlations ($P < 0.05$) with absolute densities (adults per vine$^{-1}$) (Table 1). Therefore, in the vegetative stage, the fifth and sixth leaves were suitable to sample *B. tabaci* adults because they had frequency of occurrence above 80%, relative variance lower than 25%, and significant correlations with absolute densities.

In the flowering plants, only the 10 most apical leaves presented frequencies of occurrence on the vines higher than 80%. From the third to tenth leaves, the relative variance of *B. tabaci* densities was lower than 25%. From the fourth to tenth leaves, the relative densities (adults per leaf$^{-1}$) presented significant correlations ($P < 0.05$). Thus, the fourth to tenth leaves of flowering melon plants are sampling units that may be used to sample *B. tabaci* adults.

In fruiting plants, the 15 most apical leaves presented frequencies of occurrence on the vines higher than 80%. From the fifth to fifteenth leaves the relative variance of *B. tabaci* densities was lower than 25% and the relative densities (adults per leaf$^{-1}$) presented significant correlations ($P < 0.05$) with absolute densities (adults per vine$^{-1}$) (Table 1). Thus, the fifth to fifteenth leaves of fruiting melon plants are sampling units that may be used to sample *B. tabaci* adults.

Therefore, the sampling units that were suitable in vegetative, flowering, and fruiting stages were the fifth and sixth most apical leaves of the vine. These were the ideal sampling units to generate a standardized sampling plan to be used for all phenological stages.

**SELECTION OF THE OPTIMAL SAMPLING TECHNIQUE**

In plants at vegetative and fruiting stages, higher densities of *B. tabaci* adults were detected using the leaf beating on a white tray technique than with the direct counting technique. The opposite occurred when the melon plants were at the flowering stage (Fig. 2A).

In plants at the vegetative stage, the relative variance of *B. tabaci* densities was lower than 25% when using both the leaf beating on a white tray technique and the direct counting technique. In the flowering and fruiting plants, the relative variance of *B. tabaci* densities was lower than 25% only when the direct counting technique was used (Fig. 2B).

Sampling time using the direct counting technique was shorter (average of 7 min per stage$^{-1}$) than with the leaf beating on a white tray technique (average of 14 min per stage$^{-1}$) in melon plants for all 3 phenological stages (vegetative, flowering, and fruiting) (Fig. 2C).

Thus, the best technique to sample *B. tabaci* adults in melon plants in the 3 phenological stages (vegetative, flowering, and fruiting) was direct counting.
DETERMINATION OF THE NUMBER OF SAMPLES FOR *BEMISIA TABACI* SAMPLING IN MELON FIELDS

In 9 of the 12 fields evaluated (75%), the differences between observed and expected frequencies of *B. tabaci* densities according to the negative binomial distribution were not significant ($P > 0.05$). Therefore, in these fields, pest densities followed the negative binomial frequency distribution. *Bemisia tabaci* densities followed the Poisson frequency distribution in only 3 of the melon fields. On the other hand, *B. tabaci* densities that followed a positive binomial frequency distribution were not observed in any of the fields (Table 2). Therefore, the ideal formula to calculate samples for the sampling plan is the negative binomial frequency distribution.

The simple linear regression of the common aggregation parameter of the *B. tabaci* densities in 12 melon fields ($K_{\text{common}}$) as a function of the values of the aggregation parameter of each crop showed a significant curve ($P < 0.05$) and a non-significant intercept ($P > 0.05$) (Table 3). Therefore, the *B. tabaci* densities in 12 melon fields showed a common aggregation parameter ($K_{\text{common}}$).

The sampling time for sampling plans with errors of 5 and 10% was higher than 1 h. When the sampling error was 15, 20, and 25%, the sampling time was less than 1 h. Therefore, 15% was the smallest error that generated a sampling plan with sampling time less than 1 h. Using this error (15%), the number of samples from the sampling plan was 72 per crop with a duration of 39 min and cost of US$5.27 per sample (Table 4).

**Discussion**

The fifth and sixth most apical leaves of the plant were the optimal sample units for the evaluation of *B. tabaci* in melon crops because they allowed more precise sampling of the pest for all phenological stages. In this context, these units allowed faster sampling because they were present in 100% of plant vines. These sample units also enabled accurate sampling, because *B. tabaci* densities displayed a relative variance of less than 25%. According to Southwood (1978), these samples were satisfactory for the determination of pest densities.
values generate feasible sampling plans. In addition, the fifth and sixth most apical leaves of the branch allowed representative samplings of pest attacks on the vine. This is shown by the fact that relative \textit{B. tabaci}
densities (adults per leaf\textsuperscript{–1}) showed a positive and significant correlation with the absolute density of the pest (adults per vine\textsuperscript{–1}). Moreover, these fast, precise, and representative samplings were observed for all phenological stages (vegetative, flowering, and fruiting) of melon plants. The preference of whitefly to attack younger leaves can be explained by the fact that these leaves present better nutritional content, besides having soft, fine cuticles, and a higher concentration of water available to the insect (Van Lenteren & Noldus 1990).

The best sampling technique to sample \textit{B. tabaci} adults in melon crops was direct counting due to the possibility of sampling the pest at all the phenological stages of plant growth. One of the characteristics that led to the choice of the direct counting technique was the fact that it enabled accurate sampling (relative variance lower than 25\%) in all situations. In addition, direct counting was faster than the leaf beating on a white tray technique for all phenological stages of the plant. The fact that a technique has a shorter sampling time indicates that its use will generate more feasible sampling plans (Southwood 1978; Gusmão et al. 2005; Moura et al. 2007).

The frequency distribution that best described \textit{B. tabaci} adult densities in melon crops was the negative binomial. This was probably due to variance of pest densities being higher than average for this characteristic (Tonhasca et al. 1994; Moura et al. 2003). Additionally, we observed that in melon crops with plants at vegetative, flowering, and fruiting stages, the variance in \textit{B. tabaci} densities was up to 74.65 and 104.00 times higher than the averages, respectively.

In this context, some authors misinterpret the frequency distribution data, stating that it represents the spatial distribution of insects in fields (Maruyama et al. 2006; Costa et al. 2010; Souza et al. 2013). However, this is not the case, because there is no relationship between frequency distributions of insect densities and the spatial distribution of these organisms in fields (Young & Young 1990; Barrigossi et al. 2001). To evaluate spatial distribution of insect populations in fields, it is necessary to locate the geographical coordinates of each sampling point and to analyze the data using a suitable statistical tool such as geostatistics (Young & Young 1998; Rijal et al. 2014; Rosado et al. 2015). However, frequency distribution data is an appropriate tool to select the formula to use in the calculation of samples for sampling plans (Young & Young 1998; Gusmão et al. 2005; Bacci et al. 2008) and population estimates (Pedigo & Buntin 1993; Heersink et al. 2016).

Table 2. Chi-squared test ($\chi^2$) results between the frequencies observed and expected by the negative binomial, Poisson, and positive binomial \textit{Bemisia tabaci} density distributions (means ± standard errors) in 12 fields of melon.

| Fields | Density | Negative binomial | Poisson | Positive binomial |
|--------|---------|-------------------|---------|------------------|
|        | $\chi^2$ | $\chi^2$ | $\chi^2$ | $\chi^2$ | df | df | df | df |
| Vegitative stage plants | 0.11 ± 0.02 | 1.07 NS | 1 | 7.62* | 1 | 892.24* | 1 |
| 2 | 0.04 ± 0.01 | 1.00 × 10$^{-5}$ | 1 | 0.01 NS | 1 | 172.84* | 1 |
| 3 | 4.49 ± 0.25 | 24.25 NS | 15 | 12,552.71* | 16 | 2.97 × 10$^{14}$ | 16 |
| 4 | 3.29 ± 0.17 | 33.42* | 13 | 7,415.55* | 14 | 2.05 × 10$^{14}$ | 14 |
| Flowering plants | 0.09 ± 0.03 | 0.81 NS | 2 | 1,657.02* | 3 | 317.00* | 3 |
| 6 | 0.07 ± 0.02 | 2.23 NS | 4 | 563.95* | 2 | 232.35* | 2 |
| 7 | 2.99 ± 0.16 | 9.11 NS | 8 | 378.26* | 9 | 5.88 × 10$^{14}$ | 9 |
| 8 | 4.95 ± 0.22 | 19.96 NS | 12 | 1,038.96* | 13 | 1.01 × 10$^{14}$ | 13 |
| Fruiting plants | 0.03 ± 0.01 | 9.81 × 10$^{-4}$ | 1 | 0.01 NS | 1 | 135.89* | 1 |
| 10 | 0.02 ± 0.01 | 5.50 × 10$^{-4}$ | 1 | 1.40 × 10$^{-3}$ | 1 | 71.42* | 1 |
| 11 | 4.72 ± 0.35 | 28.14* | 12 | 3,012.50* | 13 | 2.99 × 10$^{10}$ | 13 |
| 12 | 2.35 ± 0.24 | 55.94* | 10 | 13,134.12* | 11 | 7.10 × 10$^{12}$ | 11 |

$^{(*)}$Not significant at 5\% probability. *Significant at 5\% probability. df = Degrees of freedom.
The existence of a common aggregation parameter (Kcommon) for B. tabaci densities in melon crops indicates that it is possible to determine a sampling plan for this pest that is applicable to all sampled crops (Bliss & Owen 1958). This is even more relevant because the fields sampled in this project were in different phenological stages (vegetative, flowering, or fruiting). Therefore, the sampling plan generated here has great potential to be adopted by farmers due to its capacity for being used throughout all melon crop stages.

For a B. tabaci sampling plan to be viable, it should present a sampling time of less than 1 h and require a small number of samples (Bacci et al. 2006; Rosado et al. 2014). The smallest error used in the calculation to sample this pest was 15%. Therefore, it is a feasible system. According to Southwood (1978), the permitted error for this type of study can be up to 25%. The number of samples obtained was 72 samples per crop with a collection time of 39 min and a cost of US$5.27 per sample. The advantage of these systems is that producers are able to obtain more reliable (smaller sampling error), representative, faster, and less expensive samples. According to Bacci et al. (2008), these sampling techniques can reduce pesticide use, cost, and application time, and preserve the ecosystem and human health. In addition to providing the farmer with environmentally friendly management options, IPM ensures greater profitability by reducing pesticide spraying (Picanço et al. 2004; Pedigo & Rice 2014).

In conclusion, B. tabaci sampling in melon crops should be performed by direct counting of pest adults on the fifth and sixth most apical leaves of the plant vine. The sampling plan is composed of 72 samples per crop. This sampling plan is feasible with a duration of 39 min, a cost of US$5.27 per sample, and can be used in plants during vegetative, flowering, and fruiting stages.

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Table 3. Analysis of variance of Bemisia tabaci adult density, sampled in 12 melon fields, to assess the dispersion parameter of the common negative binomial distribution (Kcommon).

| Sources of variation | df | Sum of Squares | Mean Squares | F    |
|----------------------|----|----------------|--------------|------|
| Slope 1/kc           | 1  | 277.85         | 277.85       | 6.77*|
| Intercept            | 1  | 7.13           | 7.13         | 0.17*|
| Error                | 9  | 369.18         | 41.02        |      |

Kcommon = 0.9134

**Not significant at 5% probability. *Significant at 5% probability. df = Degrees of freedom.**

Table 4. Number of samples, time involved, and cost of sampling Bemisia tabaci adults in melon plants as a function of the maximum error admitted in this process.

| Error (%) | Number of Samples | Time | Cost (US$) |
|-----------|-------------------|------|------------|
| 5         | 644               | 5 h 51 min | 46.87     |
| 10        | 161               | 1 h 28 min | 11.80     |
| 15        | 72                | 39 min   | 5.27       |
| 20        | 40                | 22 min   | 3.00       |
| 25        | 26                | 14 min   | 1.94       |
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