# The Maximum Number of Short Days Required for Normal Flowering in the Maize Subspecies

Karl, J. R.

Allegany, New York, USA.
jrk36@cornell.edu

## ABSTRACT

It is difficult to work with tropical maize because its reproductive parameters are poorly known due to its night-length reactivity. To improve the science, the number of long nights required for normal reproduction in the most-reactive strain of the subspecies were found via planting the strain in a New York field and subjecting it to either 30, 35, 40, 45, 50, or 55 13-hour nights when the fifth leaf became visible. The results showed that 50 nights is the requirement, as flowering was the same beyond that. This 50th long night was on the 68th day of growth, at which time the plants were .9 m (3 ft) tall, had 12.5 protroded leaf collars, and the 18th leaf, the ear leaf, was visible. Stands receiving more than 50 long nights were 3.3 m (11 ft) tall, with 18 leaves below the ear and 6 above. Pollen began when plants were 3-3.5 months of age, whereas silk began at 3-4 months, with proterandry ranging from 1 week to 1 month. With 45 nights, pollen may be delayed 1 week, and silk ½ month, whereas 40 nights will delay pollen 1 month. Plants given only 35 long nights will be sterile, and it is by this time that the tassel has been initiated. The short-night reaction returns at 30 nights, confirming the greater reactivity of the chosen strain over established strains. Knowing this maximum requirement adds substantial certainty to tropical maize work, which never before existed.

## INTRODUCTION

Most maize races must experience a certain number of long nights in order to reproduce normally (JR Karl, manuscript in preparation). The requirement is known for only a few strains, and it is about the same, ~ 25 nights (Emerson, J Heredity 15: 41-48, 1924; Coles et al., Genetics 184: 799–812, 2010). Even though these scant findings suggest that the requirement is ~ 25 nights (i.e., long nights) for most of the short-night reactive maize varieties regardless of origin, much greater factual support is necessary as a basis for experimental studies. Establishing the limits for the tallest strain of the subspecies (dominating in both long-night height and short-night reactivity) would dispel the uncertainty and offer a way to guarantee normal reproduction in any tropical maize.

## MATERIALS AND METHODS

Tall strains of the Tehua race have proven to be a candidate for the tallest material of the subspecies (Wellhausen et al, Races of maize in Mexico, Cambridge (MA), Bussey Institute of Harvard University, 1952; Stevenson, Crop Sci 12: 864-868, 1972; Goodman and Castillo-Gonzalez, Crop Sci 29: 853-861, 1989; Sanchez and Goodman, Econ Bot 46(1):72-85, Maydica 37:41-51, 1992; Sanchez et al, Econ Bot 54:43-59, 2000; Karl, MNL 86:3-4, 2012). Seed of one such strain, Wellhausen's Chiapas 234, was isolated by crossing two plants. A stand composed of groups of four plants was cultivated, imbibition May 5$^{th}$, *in field* under the natural, short *night length* of New York in 2012. As it appears (Tollenaar and Hunter, Crop Sci 23: 457-460, 1983; Sanchez and Goodman, 1992 a, b) that maize begins "reading" night length by the time that the fifth leaf tip is detected protruding from the crotch of the preceding two leaves, when the plants reached such a stage (the 18$^{th}$ day of growth in this study), they were covered each night to extend the night length, so that one group (four plants) was grown in 30 long nights (13 hr/11-hr day, surmised to be a moderate long-night regime), another group in 35, and so on, up to a group receiving 55 nights. There were four plants for each treatment except for the *55 night* with 36 plants. When the treatment ended for a group, the

plant age, height, and number of protruded leaf collars were noted. Following each group's treatment, the plants continued development under the natural, seasonal night length of New York.

RESULTS AND ANALYSIS

When tassel protrusion approached completion, it was clear (Figure 1) that 50 nights was the requirement -- the subspecies limit. Plants receiving ≥50 long nights were normal. This is 20 more nights than that in regular tropical maize, which reinforces the idea of Tehua being regarded as the tallest.

From the notes that were made on that particular treatment, the plants were 3 ft tall, and 12.5 leaf collars (18 leaf tips) protruded at the completion of the long nights (68$^{th}$ day of growth/2 mo 1 wk). These data may be used as (approximate) alternatives to monitoring the quantity of long nights (Figure 2).

The normal stand was 11 ft tall, had 18 leaves below the ear, and 6 above (total 24; vs. 40 below ear in short night). Pollen shedding began from the 92$^{nd}$ to 106$^{th}$ day of growth (3-3.5 mo), and silk protrusion began from the 97$^{th}$ to 133$^{rd}$ day (3 mo 1 wk - 4 mo 1 wk), with a proterandry range from 5 to 27 days (1 wk – 1 mo; Figures 3, 4). These ranges include a plant that was relatively normal, though it silked 7 days later than the last of the group, possibly due to experimental error, or to the plant requiring slightly more than 55 nights.

Half of the 45-night plants began pollen shedding within the normal range, though only one began to silk in the normal range. The rest of the plants began pollen shedding 1 week late and silk protrusion 2.5 weeks late. Consequently, 45-night treatment may be considered a method to establish delayed plants for nicking/preserving late genotypes of the population. One plant did not silk, though it had an ear shoot and was killed by freezing temperatures. Growth in the experiment was ended by a freeze on October 13, which was the 160$^{th}$ day (5 mo 1 wk), though even September was too cool for normal growth. The 45-night stand may have been slightly taller. This would have been due to a dynamic that makes the internodes longer; the greater height would not have been due to more leaves, because the leaf quantity was the same as in longer treatments (Figures 5, 6).

Even the 40-night treatment plants produced pollen, though it was now not just 1 week, but 1 month late. Accordingly, this finding could be used to nick plants if, e.g., *delayed flowering* or *Leafy* were added to the population. (Two mutant segregants in an F2 of a Montana (montanya)-race accession Ecuador 689 x *delayed flowering* stock N2461 cross began to shed pollen ≥1.5 mo (5-7 weeks) later than 10 wild-type segregants (wild type ≤3 mo vs. mutant ≥4.5 mo (126-142 d)); and 4/5 mutant segregants of a third back-cross, tallest-tropical (94%) + *Leafy* strain began to shed pollen (protogyny 2 days) ~1 mo later than 9 wild-type segregants (3.5 mo wild type vs. ~4.5 mo mutant, excluding one 5.5 mo)). However, even if ear shoots had appeared in this 40-night treatment and issued silk (Figure 3: 135 days pollen + (30 days) proterandry), this could not have been indexed because of the short season (160 day/experiment parameters).

The 40-night treatment was also the first in which mutilation appeared (Figures 5, 6). It entailed the whole plant, resulting in a sterile tassel for one of the four plants (tassels were nubs, leaves were narrow and creased). This treatment may also have produced taller than normal plants, like the 45-

night treatment, though in the 40-night treatment it would have been due to an extra leaf (25 leaves in treatments now), as the internode length appeared to have unexpectedly returned to normal. This isolated the extra length/height of the 45-night treatment as an idiosyncrasy.

In the 35-night treatment, the mutilation was severe, such that there would be full sterility.

In light of the progressive increase in maturity as the quantity of long nights decreased despite a marginal change in leaf quantity, this experiment accentuates the fact that short night lengthens maturity by physiologically slowing growth, regardless of whether it further lengthens maturity by invoking the production of additional leaves.

Thirty nights is when the short-night response began returning (≥50% of plants), and in affected plants, the leaf quantity was normal for short night, approximately 45; thus, the effect seen in this experiment was almost perfectly qualitative (qualitative with high fidelity; i.e., for night *quantity*, not night *length*). This observation also establishes that this tallest material may show an unmitigated short-night response transcending traditional (25-30

night) treatment. Accordingly, the phenomenon in these plants introduced the first possible sign of increased stem diameter and coincident decrease of internode length. The remaining plants "segregated" as 35-night plants.

In the short-night plants, the whorl nodes were fused on one side, and thus did not fully separate (Figure 8). This similarly happened in a *leafy* (ear "tabs" visible) plant that developed entirely under short night (the newest leaf of the whorl was 34 ft; dissection revealed the plant's total number of leaves to be 76, followed with a 9-inch tassel that resisted protrusion; Figure 7). The phenomenon is a manner in which the leafy phenotype can be expressed. It explains the stagnation of growth, when many extra, full leaves develop (Figure 6), yet the internode elongation cannot separate them (because their nodes are moderately fused).

When Chiapas 234 is grown under long night, the qualitative nightlength reaction is precluded by 35 long nights (1 mo 3 wk growth), yet quantitative leaf addition lingers through 40 long nights, ended by the tassel before 45 long nights (2 mo growth) (Figure 9, 10). Fifty nights ensure a normal pollen and silk date; it is ironic that these final required long nights correspond with protrusion of the ear leaf from the whorl.

Other tall races appear to have the same requirements as Chiapas 234 or less. However, there are two qualifications for that statement: one concerns finicky flowerers that require constant or increasing night length beyond initiation, such as Montana; the second concerns heterosis between the tall strains in which the night-length reaction has proven to be untempered in tall-Tehua crosses (Karl, MNL 86:3-4, 2012), in which case they attain an advanced stage of development while having undergone fewer (an inadequate number of) long nights. For an example, a plant (ostensibly) of the Montana race received a 30-night treatment but reproduced just like the 40-night 234 plants (rear plant in Figure 6).

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**Figure 1. Chiapas 234 maize in rows treated with different numbers of long nights** (designated in photo). Fifty nights or more are equally effective, 45 nights appears to be at the boundary, and 40 nights is (contextually) ineffective.


**Figure 2.**

MAIZE PLANT SIZE when
REPRODUCTIVE competence is achieved.

High-end parameter.
Tallest race of the subspecies.
Tehua strain Chiapas 234.

J.Karl 2012

| Nights | Height | | Collars | | Leaves | Age: Months | Weeks | Days | Total |
|---|---|---|---|---|---|---|---|---|---|
| 30 | 25 | (10") | 8.5 | 4 | 12.5 | 1 | 2 | 4 | 48 |
| 35 | 29 | (11.5") | 9 | 4 | 13 | 1 | 3 | 2 | 53 |
| 40 | 52 | (1'8") | 10 | x | [14] | 1 | 4 | 0 | 58 |
| 45 | 76 | (2'6") | 11 | 5 | 16 | 2 | 0 | 3 | 63 |
| 50 | 91 | (3') | 12.5 | 5 | 18 | 2 | 1 | 1 | 68 |
| 55 | 129 | (4'3") | 14.5 | x | [19.5] | 2 | 1 | 6 | 73 |

difference

Nights: qty of long nights
Height: cm to crotch of 1st and 2nd-newest leaves
Collars: qty of protruded leaf collars/ligules
Leaves: qty of protruded leaf tips
Age: plant age
Total: days from imbibition

**Figure 3.**

# QUANTITY of *LONG-NIGHTS* REQUIRED for REPRODUCTION in the TALLEST STRAIN of the *MAIZE* SUBSPECIES

Chiapas 234, Tehua Race

J.Karl 2012

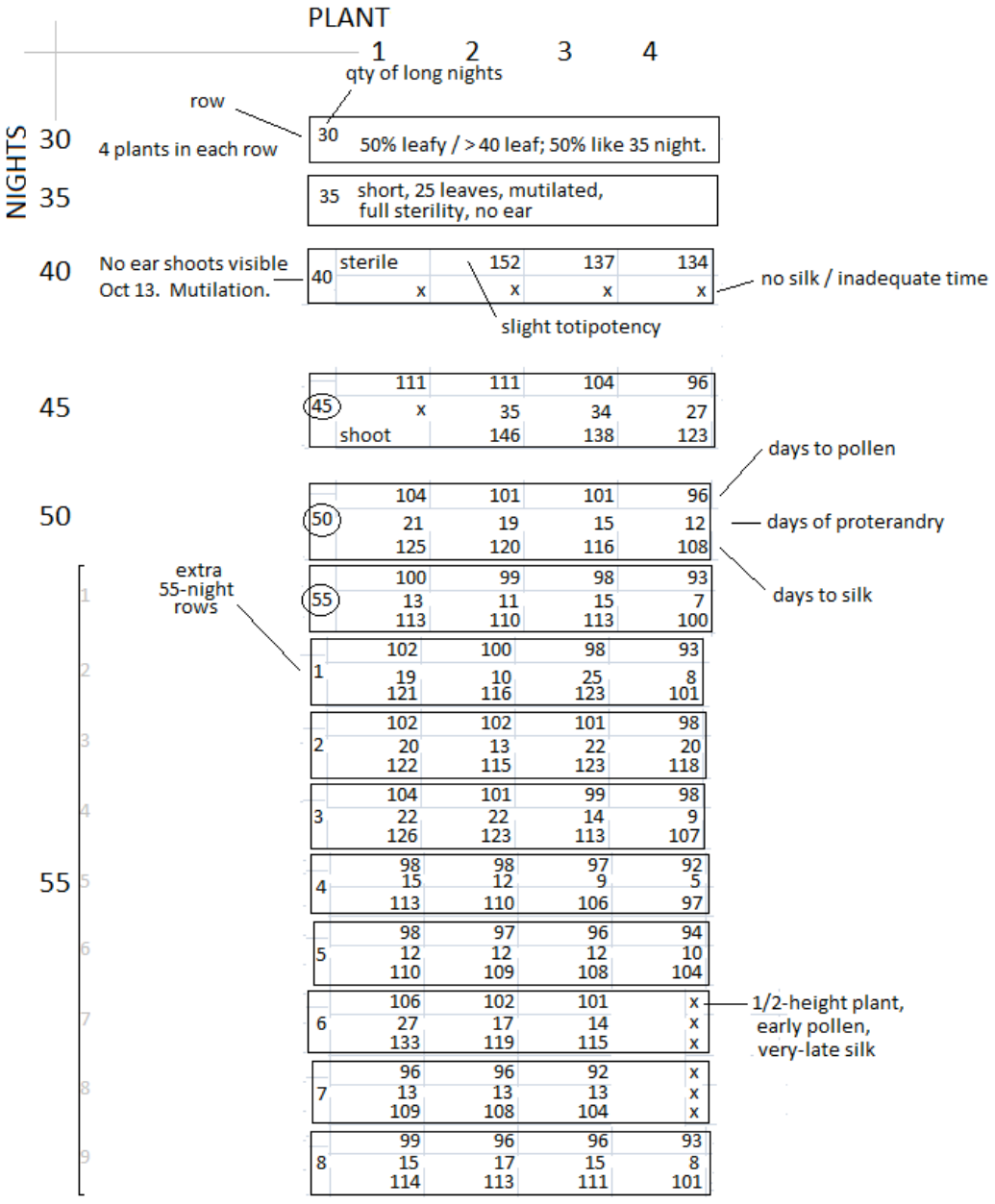
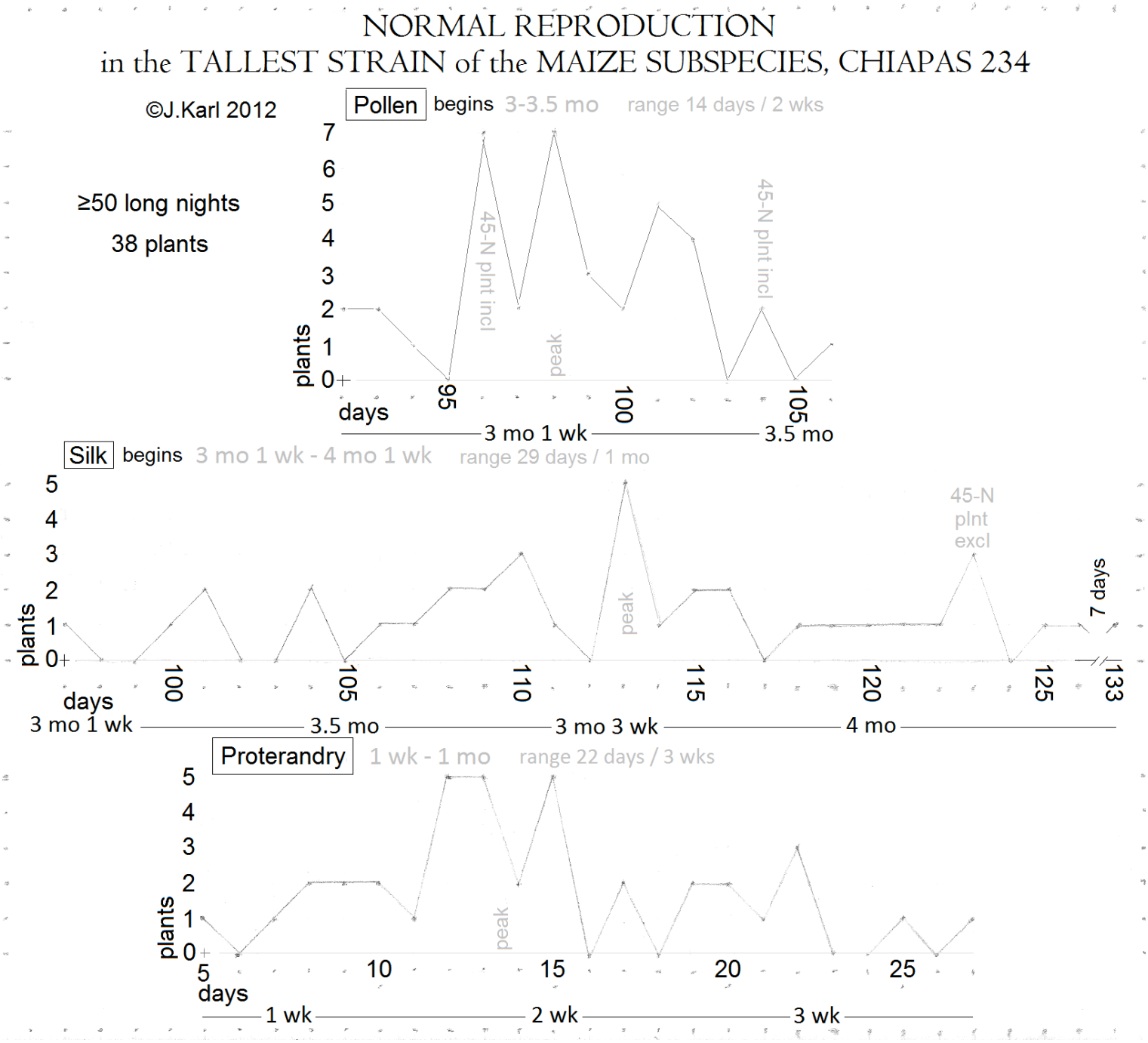


**Figure 4.**



NORMAL REPRODUCTION
in the TALLEST STRAIN of the MAIZE SUBSPECIES, CHIAPAS 234

≥50 long nights
38 plants

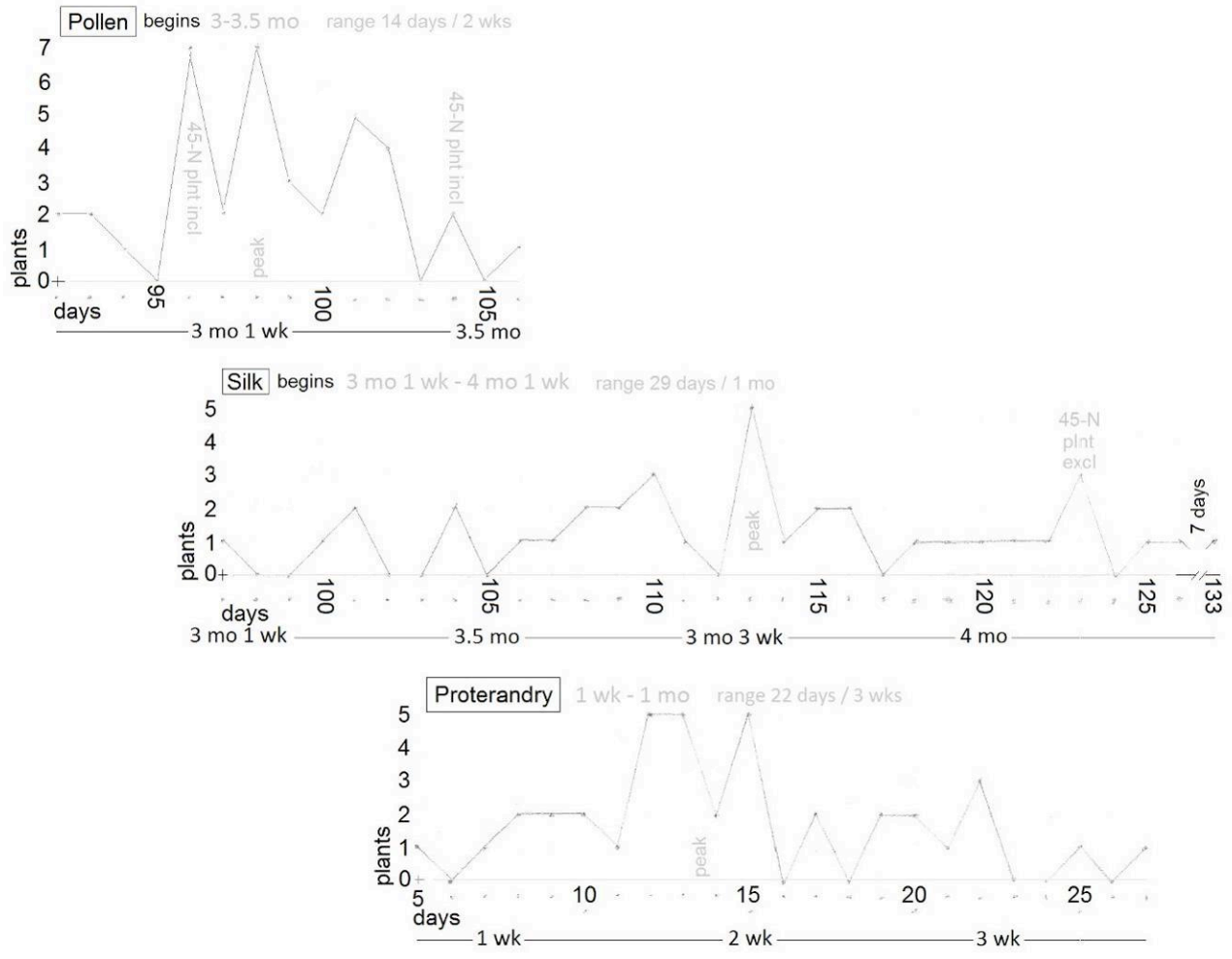


**Figure 5. Chiapas 234 maize rows treated with different numbers of long nights** (designated in photo). The 45-night row is possibly taller than normal (≥50 nights) due to longer internodes, not to more leaves. The ≤40-night rows exhibit mutilation (e.g., longitudinal leaf creasing).

**Figure 6. Chiapas 234 maize in rows treated with decreasing numbers of long nights** (designated in photo). Rows ≥50 nights were normal. Row 45 is possibly taller than normal due to longer internodes. Row 40 is possibly taller due to an extra leaf, as its internodes were normal length. There is also heavy mutilation (e.g., leaf and tassel) of the 40- and 35-night treatments.

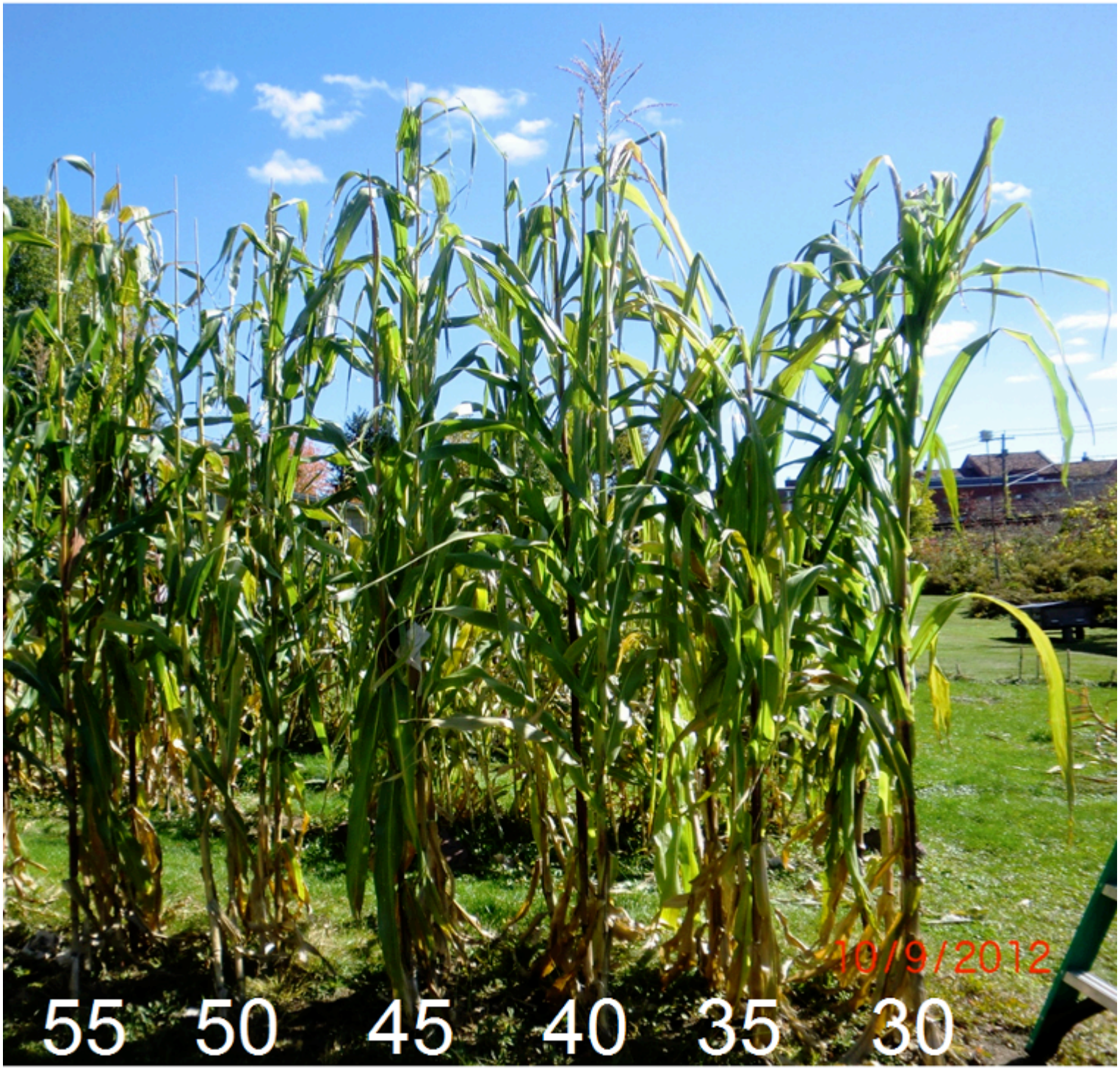


**Figure 7.** Short-night induced mutilation of the "leafy" phenotype in maize, in which nodes are fused, resulting in a much greater number of developed leaves than distinct nodes (e.g. 20; 56 nodes, 76 leaves; the multiplicity occurring only above node 50). Genotype: tall Tehua strain Chiapas 234 background. There is a corkscrew in this example (chirality alternates in segments removed above), whereas a second occurrence is illustrated in Figure 8.


**Figure 8.**

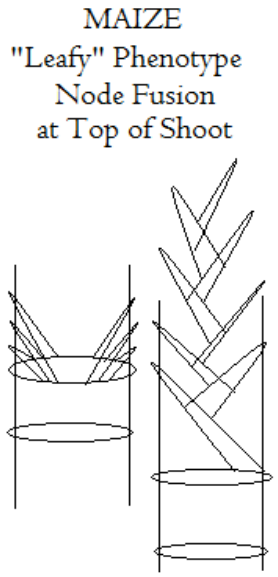



**Figure 9.** Quantity of long nights verses leaf quantity & internode length (height of leaf collar 15) in the most-reactive strain of the maize subspecies.

| | nights | | | | |
|---|---|---|---|---|---|
| | 50 | 45 | 40 | 35 | 30 |
| leaves | 23 | 23 | 25 | 26 | 27 |
| | 24 | 24 | 25 | 25 | x |
| | 24 | 24 | 25 | x | x |
| | 25 | 25 | 26 | x | x |
| | 3 | 4 | 3 | 3 | 2.5 |

height (ft) of leaf collar 15

**Figure 10**. Life map of Chiapas 234, the most nightlength-reactive strain of the subspecies.