Phenology and Biomass Production of Adapted and Non-Adapted Tropical Corn Populations in Central Iowa

Pedro Alexander Infante,* Ken J. Moore, Andrew W. Lenssen, Sotirios V. Archontoulis, Paul Scott, and Shui-zhang Fei

ABSTRACT
Biofuel production in the midwestern United States has largely focused on corn (Zea mays L.) grain for ethanol production and more recently, corn stover for lignocellulosic ethanol. Tropical corn refers to corn adapted from tropical regions. Growing tropical germplasm in temperate environments is not attractive for grain yield, but shows promise for biomass production through taller and more vigorous plants with thick stems and long leaves. The comparison between tropical corn populations and their temperate adapted counterparts with a focus on biomass production has not yet been explored under Iowa growing conditions. This study refines the accuracy of the leaf collar method, and characterizes the adaptation of the populations by evaluating crop development and biomass production. Therefore, field trials were established in central Iowa during the 2014 and 2015 growing seasons. Adapted and non-adapted versions of three populations, Tuxpño, Suwan, and Tuson, were planted at three dates to evaluate their phenology and potential for biomass production under midwestern U.S. conditions. Plant height correlated well with vegetative development and total biomass. Adapted tropical corn had advanced grain development, while non-adapted tropical corn had advanced vegetative development and greater biomass yield. Non-adapted tropical corn flowered later, had 22% greater total biomass on average (4.6 Mg ha⁻¹ greater than adapted), and had 43% greater non-grain biomass on average, up to 20 Mg ha⁻¹ (6 Mg ha⁻¹ greater than adapted), while adapted populations yielded more grain. Non-adapted tropical corn has a high potential as feedstock for Gen2 biofuels in central Iowa.

Core Ideas
- The accuracy of the Leaf Collar Method was refined.
- Tropical corn adaptability investigated in central Iowa.
- Non-adapted tropical corn had greater non-grain and total biomass as compared to their non-adapted counterparts.
- Taller tropical corn populations had the greatest biomass.
- Greater non-grain biomass occurs at the expense of grain yield.
- Tropical corn biomass has potential as Gen2 biofuel feedstock.

doi:10.2134/agronj2016.11.0666

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and BS29 for Suwan (Hallauer, 1994); likewise, the 11th cycle of Tuson formed BS39 (Hallauer and Carena, 2016). The “B” in the registration is the national identifier of lines from Iowa, and the “S” indicates it is a synthetic population. All original and mass-selected cycles (C0–C5 for Tuxpeño and Suwan; C0–C11 for Tuson) were evaluated at five Iowa locations to verify the effect of the selection made by early flowering, measuring differences in silk emergence, anthesis silking interval (ASI), grain yield, height, and lodging (Hallauer, 1999; Hallauer and Carena, 2016). Research on the possible use of adapted and non-adapted tropical corn for biomass is necessary to realize the full potential of these populations.

Compared to adapted germplasm, non-adapted tropical populations under temperate conditions have longer growth cycles, greater sensitivity to photoperiod, greater total leaf number, delayed flowering, later physiological maturity, and higher grain moisture at harvest (King et al., 1972; Kiniry et al., 1983a; Oyervides-García et al., 1985; Manrique and Hodges, 1991; Hallauer, 1999; Coles et al., 2010). The features of tropical germplasm produced in temperate environments are well known to be unacceptable from a grain yield perspective. However, taller and more vigorous plants with thick stems and longer leaves, as found in tropical corn, could be attractive from a biomass yield standpoint (Chen et al., 2013). Several studies positively relate morphometric measurements such as height and stalk diameter to vegetative growth and forage biomass (Freeman et al., 2007; Pordesimo et al., 2004).

Delayed flowering in tropical corn grown in temperate regions has been found in several studies (King et al., 1972; Kiniry et al., 1983a; Oyervides-García et al., 1985; Coles et al., 2010). Corn populations adapted to 12 to 14 h day-lengths flowered later under longer day-lengths (14–16 h), representative of temperate regions (King et al., 1972; Oyervides-García et al., 1985; Coles et al., 2010). Additionally, introduced tropical populations had 15 to 21 d of delay in grain development compared to populations adapted to Iowa environments (Hallauer, 1999). Furthermore, King et al. (1972) reported a difference of 12 d between adapted and non-adapted strains in average mid-disk date for Tuxpeño.

As reported by Kiniry et al. (1983a), photoperiod sensitive corn, such as tropical corn, has delayed tassel initiation time when grown under long day conditions. The photoperiod before tassel initiation influences leaf number. The later the tassel initiation, the greater the leaf number. Thus, tropical corn grown under long day conditions might have an increased leaf number. Corn leaf number is no longer sensitive to photoperiod after tassel initiation (Ellis et al., 1992).

Although photoperiod effects on corn are extensively studied before tassel initiation, Kiniry et al. (1983b) reported continued photoperiod effects after tassel initiation. These effects after tassel initiation are positively correlated to the duration between tassel initiation and tassel emergence (tasseling). Increased day-length after tassel initiation increased this tassel initiation-to-tasseling duration (Ellis et al., 1992). As concluded by these authors, photoperiod sensitive corn remains also sensitive to photoperiod for some time after tassel initiation.

The delay in development conveys effects on growth and grain yield. The reduction in grain yield and number of kernels per ear is related to the length of the anthesis-silking interval (ASI or silk delay) (Edmeades et al., 1993). Edmeades et al. (1993) found a 90% reduction of grain yield when the ASI changed from 0.4 to 10 d. As suggested by Ellis et al. (1992), there are cascade effects in photoperiod sensitive corn grown under long day conditions, which include increased leaf number, greater leaf area duration, and consequently more photosynthesis per day produced. Delayed flowering and more specifically delayed shoot maturation could lead to the accumulation of more vegetative growth at the expense of grain yield (Manrique and Hodges, 1991; Pordesimo et al., 2004).

According to White et al. (2011), tropical corn has reduced grain formation because it accumulates a large amount of extractable sugar, primarily sucrose, glucose, and fructose, in the stalk. Therefore, tropical corn accumulates more sugar during grain filling because kernel set is poor and barrenness often occurs. Furthermore, corn germplasm with delayed flowering, as found in tropical corn, would compensate for the reduction of grain with more vegetative biomass (King et al., 1972; Pordesimo et al., 2011; Chen et al., 2013). The source greatly exceeds the sink, especially when grown at longer days.

Reported results show that delayed flowering, such as found in non-adapted tropical corn, was antagonistic to grain production (King et al., 1972; Pordesimo et al., 2011; White et al., 2011; Chen et al., 2013). However, even if corn has been historically cultivated mainly for grain, and all fertilization and water recommendations have been calculated for this purpose, biomass accumulation in tropical corn may be useful for bioenergy, meaning studying corn’s overall potential beyond grain, remains relevant (White et al., 2011; Chen et al., 2013). This potential can be better understood through further refined study of phenology and vegetative development of tropical populations. Little emphasis has been given to corn biomass production, and the requirements for vegetative growth production centered on biomass have not been studied in depth, or from a phenological standpoint (Carpita and McCann, 2008). Moreover, the comparison between tropical corn and corresponding adapted strains with a focus on biomass production has not yet been explored under Iowa day-length conditions.

Currently, the majority of ethanol is being produced from corn grain, by starch-based ethanol production, according to the Renewable Fuel Standard (RFS). Tropical corn biomass has potential as Gen2 biofuel feedstock (Wilhelm et al., 2011), but little is known about the phenological development when grown for biomass. The objective of this research was to investigate the potential of tropical corn populations for biomass production by means of studying crop development. Phenological and physiological aspects of this study will identify the suitability of these sources as biomass feedstock for cellulosic biofuel production.

**MATERIALS AND METHODS**

**Site Description and Plant Materials**

A field study (Fig. 1) was established at the Sorensen Research Farm of Iowa State University in Boone County, IA, located at 42°02’ N and 93°46’ W during the 2014 and 2015 growing seasons. Day-length conditions of 15 to 14.9 h are typical of central Iowa when corn is first receptive, around V5 and V8 for tropical corn (Kiniry, 1991). Weather data were collected from the station BOOI4 (Table 1), located on the same farm, and the data were compiled from the Iowa Environmental Mesonet (ISU AgClimate network). The experimental design was a split-split plot with three replications. The first split was planting date, and the second split was adaptation. Two weeks before planting, N fertilizer was applied uniformly at a rate of 190 kg N ha⁻¹. Three planting dates were used each year: first planting (7 May 2014 and 13 May 2015),
second planting (20 May 2014 and 27 May 2015), and third planting (3 June 2014 and 9 June 2015). Four-row plots of 3 by 4.2 m were laid out in the field, and corn was planted with a four-row planter (Almaco, Nevada, IA) at a seed rate of 80,000 plants per hectare and row spacing of 76 cm.

Tuxpeño, Suwan, and Tuson were the tropical corn populations chosen based on their importance in tropical corn breeding programs (Hallauer, 1999; Teixeira et al., 2015). The adapted strains had five cycles of selection for adaptation (C5), and the non-adapted strains had one (C1). Populations were provided by the North Central Regional Plant Introduction Station (NCRPIS, USDA) in Ames, IA. Since early silking was the main criterion of selection, the adapted populations were less photoperiod sensitive, showing inherent earliness and fewer leaves than non-adapted populations when grown in central Iowa conditions. The seed lots were treated with thiamethoxam at 0.48 mg kernel$^{-1}$, Maxim Quattro at 16 mL 80,000 seeds$^{-1}$, and colorant at 10 mL kg$^{-1}$ of seed. To avoid border and shading effects due to anticipated differences in canopy height, tropical corn of an average height was planted between adapted and non-adapted strains.

**Plant Measurements**

Leaf stage was measured using the leaf collar method (LCM), which relies on visual indicators for staging vegetative (V) development (Abendroth et al., 2011); other visual indicators and destructive methods were used for reproductive (R) development. Vegetative development stages are defined by the number of collared leaves, while R stages are defined by visual indicators of kernel development (Fig. 1). The main V stages in this method are VE-emergence, V1-first leaf, V2-s leaf, V3-third leaf, Vn-nth leaf, and VT-tasseling. The main R stages are R1-silking, R2-blister, R3-milk, R4-dough, R5-dent, and R6-physiological maturity (Ransom, 2013). Specific descriptions of each of the stages for the LCM are explained by Abendroth et al. (2011). Each stage of development was achieved when 50% or more of the plants in a plot reached a specific stage (Abendroth et al., 2011; Ransom, 2013). For instance, emergence is defined as the stage when at least 50% of the planted seeds have emerged above the soil surface, and physiological maturity occurs when at least 50% of randomly selected ears of the sample have kernels with a maximum accumulation of dry matter, as determined by a 35% grain moisture (Tsimba et al., 2013).

The number of days to progress from one stage to the next varies for both vegetative and reproductive development (Abendroth et al., 2011), and thus it is necessary to fine-tune to 0.25 stages to refine and narrow in. Therefore, a novel refinement to the LCM for corn staging was implemented to enhance its accuracy, by division of each stage into quarter intervals. The criteria to measure phenology used each collar development as follows: (i) at a 0.25, when precedent collar leaf was fully developed and emerging developing leaf collar begins to show an angle noticeable at touch, (ii) at a 0.50, when the emerging collared leaf has a clearer angle at touch and it is evidently rolled close to the precedent leaf, (iii) at a 0.75, when the emerging collared leaf has a clear open V form due to the overlapping of the auricles, and (iv) appearance of fully collared leaf when auricles are evident, visually and tactiley (Fig. 2).

![Table 1](image)

**Table 1.** Average monthly temperature, accumulated monthly growing degree days (GDD), precipitation, and solar radiation for 2014, 2015, and the 30-yr average in Ames, IA.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature</th>
<th>GDD10</th>
<th>Precipitation</th>
<th>Solar radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>10</td>
<td>mm</td>
<td>[MJ m$^{-2}$]</td>
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<td>182, 192, 196</td>
<td>101, 109, 112</td>
<td>19, 16, 19</td>
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<td>June</td>
<td>21, 21, 21</td>
<td>347, 346, 340</td>
<td>233, 169, 123</td>
<td>20, 20, 21</td>
</tr>
<tr>
<td>July</td>
<td>18, 22, 23</td>
<td>344, 392, 410</td>
<td>57, 150, 108</td>
<td>21, 21, 22</td>
</tr>
<tr>
<td>Aug.</td>
<td>22, 21, 22</td>
<td>389, 340, 377</td>
<td>182, 204, 115</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Sept.</td>
<td>17, 21, 17</td>
<td>217, 318, 239</td>
<td>102, 129, 70</td>
<td>15, 16, 15</td>
</tr>
<tr>
<td>Oct.</td>
<td>11, 12, 10</td>
<td>70, 88, 78</td>
<td>86, 32, 64</td>
<td>11, 11, 10</td>
</tr>
<tr>
<td>Nov.</td>
<td>0, 5, 2</td>
<td>1, 26, 9</td>
<td>25, 62, 51</td>
<td>7, 7, 6</td>
</tr>
</tbody>
</table>

**Fig. 1.** Phenology methods design for weekly corn staging.
Because some of the tropical populations start flowering very early or late, phenology measurements were carefully collected during the growing season. Ten contiguous plants were randomly chosen from two central rows (five plants per row) of each four-row plot. Phenological development was determined every 8±2 d, depending on the developmental stage and weather conditions.

For the V period, nondestructive phenology determination was performed by counting the leaf collar number of each of the mentioned ten plants during the growing season (Fig. 1). Similarly, the interval from visible silks to first extruding anthers was measured. Since leaf number is a critical point for V staging, and leaves senesce with plant age, plants were marked on the 5th, 10th, and 15th numbered leaves using a commercial white scuff cover (Kiwi, Johnson & Son, Racine, WI) at approximately 10 cm from the appropriate leaf collar.

The height of three plants was measured after R development was initiated and V development remained fairly stable. This occurred later, up to 130 d after planting (DAP), and was determined by measuring the distance from the ground surface to the height of the final leaf collar (Fig. 3 and 4).

Reproductive development was recorded around 50 DAP when tasseling had begun, starting with nondestructive estimation (Fig. 1). Nevertheless, the staging of R development after R2 was measured accurately by the development of mid-kernels, which are underneath the husk (Abendroth et al., 2011). Therefore, a separate destructive sampling was made to determine kernel development for all treatments.

Once the R2 stage was reached, the ears of three contiguous plants were randomly chosen (in between the vegetative phenology sample and the 1-m border row) from the two central rows of each plot and were collected weekly. The number of kernels, developmental stage, ear fresh mass, and ear dry mass were recorded until the R6 stage.

For destructive sampling (Fig. 1), after 84 DAP (2014) and 75 DAP (2015), ears were sampled weekly until physiological maturity and were immediately stored in a refrigerated room at approximately 4°C. The fresh mass of ears and kernels plus cob was measured. Kernel development was determined as per Abendroth et al. (2011) including division of each R stage mean into quarter intervals (Fig. 5). Subsequently, at R6, the kernels were removed with the use of a bell corn crank sheller (C.S. Bell Co, Tiffin, OH). Kernels from ears harvested before the R6 stage were separated from the cob with the sheller after drying. Cobs and kernels were placed in separate bags and then oven dried at 60°C until reaching constant mass. Finally, the dry mass of kernels and cobs was recorded for further analysis.

Within the center rows of the four row plot, 1 m was manually harvested at an approximate distance of 1.5 m from the edge of the plot. The area harvested coincided with the plants that were reused for vegetative phenology measurements. The fresh weight of the 54 plots was recorded, and organs (leaves, stems, husks, cobs, and grains) were harvested from three randomly chosen plants and bulked. Every organ was separated in the field, with its fresh weight recorded and dried at 60°C until a constant dry weight was achieved. Samples were reweighed for dry mass and transported to the laboratory for further processing.

For kernel development, a manual pressurized cutting test was conducted on the middle kernels of each ear for all the ears collected (Fig. 5). The R3 stage was determined when premature kernels burst easily, R4 when premature kernels were difficult to burst, R5 when kernels did not burst and slicing the kernel with a knife was needed to visualize starch layer content, and R6 when kernels fractured under pressure because of lower water percentage, meaning maximum starch accumulation had occurred.

As described above, each V and R stage was divided into quarters to classify the phenology more precisely (Fig. 2 and 5). In the case of V, classification was based on leaf collar appearance, and in the case of R, classification was based on independent criteria according to the main stage, such as the length (by percentage) of the silk for R1, ease of bursting for R3–R4, and identification of the starch layer for R5 (Fig. 5).

Experimental Design and Data Analysis

The experiment was analyzed as a split-split plot with three replications during 2014 and 2015. The first split was planting date which had three levels to account for a range of diverse environments to estimate parameters associated with the photoperiod, and the second split was adaptation which has two levels (C1 and C5) for Tuxpeño, Suwan, and Tuson. Data were analyzed using the PROC GLIMMIX procedure of SAS (SAS for Windows 9.4, SAS Inst., Cary, NC). Repeated measures analysis was used to account for potential correlations among weekly measurements over the course of the growing season. Mean comparisons were made using t tests to examine differences in adaptation, germination, and planting dates. Vegetative development, reproductive development, dry matter yield, and plant height were analyzed using an α value of 0.05 (Table 2).

![Fig. 2. Leaf collar method with leaves 2, 3, and 4 indicated. The vegetative stage reported under each photograph is based on characterization of the non-collared upper leaf with quarter accuracy. Photo credit: Lori Abendroth, Iowa State University.](image-url)
RESULTS AND DISCUSSION

To evaluate the possibilities of increasing biomass yields, the potential of tropical corn grown under Iowa conditions was investigated by studying the phenology of adapted and non-adapted populations. Accuracy of Leaf Collar Method (LCM) was enhanced, contributing to the detection of phenological differences. In this research, the refinement consisted of the division of each mean stage into well-defined quarter intervals as mentioned above (Fig. 2 and 5). In addition to LCM, a complementary destructive sampling harvest was added because of the uncertainty when recording the precise staging from R2 to R6 solely through external examination of the ears (Fig. 1). Grain contribution to total biomass is not relevant yet because of the negligible lignocellulosic grain contents. Tropical populations do not produce the high yields necessary to be competitive in a grain market. Nevertheless, grain is a pertinent component in the comparison of each pair of populations, in terms of biomass distribution and the photoperiod effect on adapted and non-adapted populations.

Vegetative development, plant height, and non-grain biomass are discussed to provide quantification of adapted and non-adapted populations as a potential feedstock for bioprocessing. In addition, reproductive development, harvest index, and grain yield

Fig. 3. Vegetative development between adapted (striped bars) and non-adapted (white bars) tropical corn for three planting dates during 2014 and 2015. Diamonds indicate the date the adapted (filled diamonds) and the non-adapted (open diamonds) plants achieved the maximum leaf collar number; final vegetative development as measured by the Leaf Collar Method was met by 120 DAP. Different letters on the top of standard error bars mean significant differences among treatments ($P > 0.05$).
Greater Vegetative Development for Non-Adapted Populations

The vegetative development of tropical corn in 2014 and 2015 is shown at the final V stage of development in Fig. 3, and Fig. 6a to 6b shows the rate of V and R development of the corn in the study. Even though visual differences among populations with differing adaptation were recorded early in the growing season, only after 990 growing degree days (GDD), were significant differences found between them, starting with vegetative development (Fig. 3, 6a–6b). Adapted strains were slightly more developed than non-adapted from 64 to 84 DAP for 2014, and predominantly followed the same trend from 51 to 74 DAP for 2015. At around 89 DAP for 2014 and by 76 DAP for 2015, adapted strains approached an asymptote, while non-adapted strains continued to develop vegetatively and reached greater final vegetative development (Fig. 6a–6b). Adapted populations were selected for early flowering, and are less photoperiod sensitive than non-adapted populations, developing inherent earliness and fewer leaves than non-adapted populations when grown under long day conditions. The present results document that differences between adapted and non-adapted populations occur progressively through the growing season.

According to the Kiniry's phenology model (1991), the rate of tassel induction for daylengths greater than 12.5 h is a response to the number of day-length hours and a genotype-specific parameter for sensitivity. Using a calculation base of 0.52 as the...
Fig. 5. Representation of the division into quarters for kernels in the R5 main stage. The black layer indicates the starch layer to be sensed by a pressure test, and the endosperm's inner white section represents kernel water content. Pressure from a standard knife is applied progressively from the top to the bottom side of the kernel to determine stage. The representation of the cross-section of kernels drawn are in a perpendicular mode to the cob axis.

Table 2. Analysis of variance for vegetative development (V), plant height (H), and total biomass in dry matter yield (DMY), and significance in response to germplasm (G), adaptation (A), and planting dates (PD) during the 2014 and 2015 growing seasons in Boone, IA. Because of the significant multiple interactions by year, all V and H comparisons were made within each year.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>V</th>
<th>H</th>
<th>DMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>5239.19***</td>
<td>8.26**</td>
<td>32.31****</td>
</tr>
<tr>
<td>Block</td>
<td>0.03</td>
<td>2.36</td>
<td>1.26</td>
</tr>
<tr>
<td>PD x Year</td>
<td>1.28</td>
<td>2.31</td>
<td>2.8</td>
</tr>
<tr>
<td>PD x V</td>
<td>1512.56***</td>
<td>0.58</td>
<td>1.61</td>
</tr>
<tr>
<td>Adaptation (A)</td>
<td>2.4</td>
<td>90.54***</td>
<td>82.47***</td>
</tr>
<tr>
<td>A x Year</td>
<td>28.65***</td>
<td>11.61**</td>
<td>2.99</td>
</tr>
<tr>
<td>A x PD</td>
<td>0.55</td>
<td>15.76</td>
<td>0.66</td>
</tr>
<tr>
<td>A x PD x Year</td>
<td>19.85***</td>
<td>3.13*</td>
<td>0.74</td>
</tr>
<tr>
<td>Germplasm (G)</td>
<td>3.84*</td>
<td>291.92***</td>
<td>14.97***</td>
</tr>
<tr>
<td>G x Year</td>
<td>82.71***</td>
<td>0.19</td>
<td>1.86</td>
</tr>
<tr>
<td>G x PD</td>
<td>3.04*</td>
<td>3.93*</td>
<td>0.36</td>
</tr>
<tr>
<td>G x PD x Year</td>
<td>10.04***</td>
<td>1.83</td>
<td>0.36</td>
</tr>
<tr>
<td>G x A</td>
<td>1.12</td>
<td>7.1**</td>
<td>2.26</td>
</tr>
<tr>
<td>G x A x Year</td>
<td>36.43***</td>
<td>1.13</td>
<td>0.52</td>
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<tr>
<td>G x A x PD</td>
<td>1.17</td>
<td>4.49**</td>
<td>0.52</td>
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<td>3.65**</td>
<td>0.88</td>
</tr>
<tr>
<td>Day</td>
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</tr>
<tr>
<td>Day x Year</td>
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<tr>
<td>Day x PD</td>
<td>45.2***</td>
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<td>67.77***</td>
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<td>G x Day</td>
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<td>G x Day x PD x Year</td>
<td>7.28***</td>
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<td>G x A x Day</td>
<td>10.77***</td>
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<td>G x A x Day x Year</td>
<td>7.97***</td>
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<tr>
<td>G x A x Day x PD</td>
<td>5.17***</td>
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<tr>
<td>G x A x Day x PD x Year</td>
<td>2.24***</td>
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* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Non-adapted populations were taller than adapted ones, as indicated by the average final number of leaves (Fig. 4). Thus, Tuson was the tallest genotype, especially the non-adapted strain, for the three planting dates (µ = 285 cm). Tuson also had the greatest final vegetative development for all planting dates compared to Suwan and Tuxpeño (Fig. 3). In contrast, Tuxpeño had the shortest height for all planting dates (µ = 227 cm), and the least vegetative development compared to Suwan and Tuson. While no prior research compares height and development of these tropical populations...
Fig. 6. Stages of development for adapted (filled bullets) and non-adapted (open bullets) tropical corn according to the Leaf Collar Method. The above figure compares the phenology averages for Tuxpeño (green circles), Suwan (orange diamonds), and Tuson (blue squares) during the (a) 2014 and (b) 2015 growing seasons. The left axis represents the stage of vegetative (V) development, and the longer lines correspond to the V part of phenology of adapted and non-adapted populations. The right axis represents the stage of reproductive (R) development, and the shorter lines correspond to the R part of the phenology of adapted and non-adapted populations.
and their adapted counterparts, this study found that plant height correlated with final vegetative development and total biomass (Fig. 3, 4, and 7).

In comparison to commercial hybrids, Wilhelm et al. (2011) measured the mean maximum plant height of 240 cm in corn grown in Ames, IA, among a wide range of germplasm. Nevertheless, those height measurements included the stalk up to the far end of the tassel, and could result in variability due to breakage or loss of tassel. The average height reported in this study is the height of the final leaf collar; therefore, variability was not a measuring problem.

First, Suwan had intermediate height among the populations, and yielded more grain (7.2 Mg ha\(^{-1}\) for adapted and 5.7 Mg ha\(^{-1}\) for non-adapted) than Tuxpeño and Tuson. Non-adapted Suwan produced a total biomass (25.5 Mg ha\(^{-1}\)) close to that of Tuson (25.9 Mg ha\(^{-1}\)), which had the greatest biomass production. This indicates that grain, in addition to vegetative biomass, was a large contributor to Suwan’s total biomass.

Second, Tuson, the tallest of the populations in this study, reached the greatest final vegetative development (Fig. 6a–6b), represented by a greater number of leaves, and the greatest stover dry matter yield (DMY). Conversely, Tuson had the least reproductive development (Fig. 6a–6b) and accumulated a relatively lower percentage of grain DMY as compared to Suwan. This DMY ratio (stover DMY/grain DMY) may offer a tool to discriminate among corn populations for their potential to produce high lignocellulosic biomass.

Finally, Tuxpeño showed less final vegetative development (Fig. 6a–6b) and the least DMY for stems, but the fastest and greatest reproductive development before the asymptote was reached (Fig. 6a–6b). Tuxpeño was the shortest population and yielded the least total biomass, whereas Tuson and Suwan, as taller populations,
had the greatest total biomass (Fig. 4 and 7). These findings indicate that plant height may be a valuable metric to predict vegetative biomass yield.

Vega et al. (2001) determined kernel number per plant to be a good predictor of growth rate during seed set. This has potential to impact total biomass. In this study, the average kernel numbers per ear for non-adapted Suwan, Tuson, and Tuxpeño populations were found to be 432, 400, and 429, respectively, and for adapted Suwan, Tuson, and Tuxpeño populations were 438, 420, and 432, respectively. These kernel numbers were positively related to grain yield, and the kernel number was higher in adapted than non-adapted populations. However, the present study found no direct relationship between kernel number and total biomass in the populations studied. Vega et al. (2001) also presented other simple morphometric variables in relation to shoot dry matter, including plant height; however, the relationship between the two was not clear from Vega et al.’s (2001) conclusions. As discussed above, this study found plant height to be a valid predictor of total biomass. This is consistent with studies involving forage biomass during vegetative and early reproductive growth (Freeman et al., 2007; Pordesimo et al., 2004). Overall, these results document that plant height is a positively correlated indicator of total biomass production. This may be a useful tool in future research to better identify highly vegetative plants with high throughput.

According to Hallauer and Carena (2016), some adapted populations of Tuxpeño, Suwan, and Tuson had progressively higher root and stalk lodging, and this result proportionally corresponded to the height reported in the present study. An additional criterion for selecting Tuson for early silking was shortness, but even the 11th cycle of selection was reported to have a combined greatest root and stalk lodging of 16% (Hallauer and Carena, 2016), meaning the C5 Tuson used was expected to be taller. The taller the population, the greater the lodging percentage. Lodging was not a significant issue for this study in terms of observed and harvested plants; less than one-third of the lodging percentage reported by Hallauer and Carena (2016) was found in this study. However, lodging was proportional to height. Minimal lodging at this plant density was fortuitous, but further research on biomass yield under central Iowa conditions should consider lodging since it is associated with tall plants.

In terms of total biomass, there were significant differences between adapted and non-adapted populations when measured at final harvest (Table 2, Fig. 7). However, planting date did not influence DMY at the end of the growing season (Table 2). Non-adapted Suwan and Tuson had the highest total DMY (Fig. 7), while Tuxpeño yielded 10 and 8% less total biomass than Tuson and Suwan, respectively. The tropical populations evaluated in this study were used to phenologically compare differing adaptations under Corn Belt conditions; literature reports a range of 7 to 13 Mg ha\(^{-1}\) stover DMY for hybrids in Corn Belt sites (Wilhelm et al., 2011), while the present study had up to 18.3 Mg ha\(^{-1}\) stover DMY for non-adapted Tuson. It can be concluded that non-adapted tropical populations are suitable for high lignocellulosic biomass yield with up to 40% more stover DMY than the maximum stover DMY previously reported for the Corn Belt. These results indicate tropical corn’s high potential as feedstock for Gen2 biofuel production.

Later Reproductive Development for Non-Adapted Populations

Differences in the initiation of reproductive development were observed between adapted and non-adapted populations, even as early as tasseling (Fig. 6a–6b). Although adapted strains had lower final V development, they transitioned to reproductive growth more quickly (≥R1; 10–15 d earlier), approximately 80 DAP for 2014 and 74 DAP for 2015 (Fig. 6a–6b). The R stages of development began comparatively late for the non-adapted populations following this trend through R6. Therefore, ears of adapted populations developed faster than non-adapted populations for all planting dates (Fig. 6a–6b). In addition, Tuxpeño populations reached the earliest final reproductive development over Suwan and Tuson for all planting dates (Fig. 6a–6b). These outcomes are consistent with the results of King et al. (1972), who reported a delay of 12 d for non-adapted Tuxpeño. Thus, reproductive development was significantly different among populations and level of adaptation (Fig. 6a–6b).

Comparing adapted and non-adapted populations at the same plant density may be misleading because of differing levels of competition due to a greater leaf area index (LAI) in non-adapted populations. According to Edmeades et al. (2000), tropical corn may exhibit increased ASI when grown in temperate regions with long photoperiods. The authors inferred the increased ASI may be caused by the increase in LAI in long days, due to the lack of adaptation to long-day environments. At flowering, non-adapted populations might have a greater canopy that would strongly compete with the development of reproductive organs, while adapted populations had fewer leaves and earlier flowering with increased probabilities to succeed in kernel production. Competition during flowering has effects on grain filling and kernel development since non-adapted corn with greater LAI and more competition would result in more kernel barrenness in ears. These effects may well account for lower harvest indexes in non-adapted populations (Fig. 7).

Harvest Index and Grain Yield for Tropical Populations

Tropical populations had different harvest index (HI) values depending on the adaptation. Non-adapted populations resulted in a lower HI, ranging from 0.20 to 0.26 HI, and adapted populations had greater HI, ranging from 0.30 to 0.41 HI (Fig. 7). The change in HI among non-adapted and adapted populations is evidence of the tradeoff between the rate of vegetative and reproductive development and growth during the adaptation (Fig. 7). Changes in HI were found for similar selection processes (Edmeades et al., 1999). Tropical populations in this study had up to 30% lower HI compared to commercial corn, which has a typical HI of 0.5 (Wilhelm et al., 2011). In addition, the planting date did not affect HI, likely because of the small day-length difference (0.1 h) when the corn first was receptive between the first and third planting dates. Similarly, there were no differences in the flowering date among planting dates, again most likely due to this small difference in day-length.

Likewise, grain DMY differed significantly by adaptation at final harvest. There were also significant differences in grain DMY between populations when measured at the end of the growing season. Grain yields of all tropical corn gradually increased with the progression of the R mean stage for both years, while kernel
number remained fairly stable. Adapted Suwan had the greatest grain DMY at 7.23 Mg ha\(^{-1}\), adapted Tuxpeño had 18% less grain DMY than Suwan, and adapted Tuson had the least grain DMY, 26% lower than Suwan. Overall, adapted tropical corn had greater grain yields than non-adapted corn, while non-adapted tropical corn had greater total biomass yield than their adapted counterpart populations (Fig. 7). Tropical populations in this study had up to 40% lower grain DMY compared to commercial corn, which has a mean grain yield of 12.7 Mg ha\(^{-1}\) under Midwest conditions (Wilhelm et al., 2011). Therefore, these populations are far from being considered acceptable for grain production; instead, they are useful for breeding programs, and this research shows tropical corn has potential as a Gen2 feedstock.

### Future Research for the Potential of Tropical Corn

The issue of high moisture in biomass at harvest is significant and related to transport costs in other dedicated biofuel crops, such as in sorghum (Sorghum spp.) cane (Rooney et al., 2007). Tropical corn populations grown under midwestern conditions, when harvested close to the first killing frost as compared to an earlier harvest, might show reduced biomass moisture. Harvesting late in the growing season might be recommended since biomass will be drier, most likely better for lignocellulosic processing and reduced grain moisture (Liu et al., 2009). Harvesting stover well after the first killing frost generally results in a more suitable moisture concentration for storage as long as precipitation does not occur.

Tropical populations grown in temperate conditions have an extended period of vegetative growth and taller stems. As a consequence, they result in high feedstock yield (Pulam, 2011). However, these populations have been planted using conventional practices of row spacing and plant density, though optimum row spacing and plant density for increased biomass production has not been investigated. Thus, further research needs to disclose better practices when considering tropical corn favoring non-grain biomass over grain production. This might produce greater biomass production for bioenergy feedstock.

Although tropical populations' grain yield is small, this grain yield component at harvest will potentially generate either issues or opportunities requiring different harvesting and processing logistics. Further studies should consider the quality of the feedstock and its implications in terms of economic value. Indeed, non-adapted tropical populations offer a significant amount of lignocellulosic biomass, but quality must be established prior to determination of economic value. There is a potential flexibility in relation to the use of the final products, since stover might have a destination in bioenergy, while grain might have potential for multiple purposes, such as feed and starch-based ethanol production.

### Conclusions

The present study found phenological differences between adaptations appeared progressively through the growing season, and the refinement of LCM accuracy contributed to their detection. Plant height correlated well with vegetative development and total biomass for tropical populations. Tuxpeño populations were the shortest, while Suwan and Tuson had greater total biomass. Suwan had the highest grain yield of the populations, while non-adapted Tuson was the tallest of the populations and had the highest non-grain biomass.

Delay in grain development as found for non-adapted strains was antagonistic to grain yield. This relationship is reflected in a low grain DMY for all tropical populations, but was even lower for non-adapted populations (Fig. 7). On the other hand, stover DMY was significantly higher for non-adapted strains for all planting dates. These results provide evidence that more accumulation of non-grain biomass might occur at the expense of grain yield for non-adapted strains due to delayed maturation, most likely attributed to a longer timeframe for photosynthesis by non-adapted strains.

Adapted tropical corn performed better for grain yield whereas tropical corn performed better for biomass yield. The choice of non-adapted tropical corn grown under central Iowa conditions results not only in plants with delayed flowering, late grain accumulation, and delayed reproductive development, but also in plants with greater biomass, vegetative development, and height. Production of non-adapted tropical corn under central Iowa conditions will produce greater biomass yields, which could be advantageous for bioenergy feedstock and other chemical substrates.

### Acknowledgments

We would like to thank Dr. Arnel Hallauer and The North Central Regional Plant Introduction Station for donating the tropical populations for this study. We would like to specially acknowledge Dr. Mark Westgate and Lori Abendroth who reviewed the manuscript with their deep and extensive knowledge, we appreciate their professional advice and support. We would also like to thank all people involved in this research not only in the field, but also in lab processing of the present study: Alison Robertson, John Shriver, Luke Hodnefield, Jérémie Bouriot, Shawnee Webb, Colin Nahkunst, Emma Haven, Danielle Wilson, Guillermo Marcillo, and Eric Spickerman.

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