Clonally Propagated and Seed-derived Papaya Orchards: I. Plant Production and Field Growth

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Abstract. Papaya seedlings segregate for sex expression as females or hermaphrodites. Typically only hermaphrodite fruit are marketed in Hawaii. The agronomic practice of growing multiple seedlings that are later thinned to a single hermaphrodite tree is wasteful of seed, labor, and resources, especially when seed is costly. We compared growth of plants propagated by the clonal methods of micropropagation or rooting vegetative cuttings versus plants initiated as seedlings and transplanted. The seedlings were either single- or multiple-planted hermaphrodites as identified by the polymerase chain reaction (PCR) or multiple-planted, thinned seedlings. The experiments were carried out in three different locations on two islands in Hawaii. Clonally propagated plants were significantly shorter than seedlings and bore flowers earlier and lower on the trunk at all locations. Stem diameter differences were not significant even though plant size was different at planting time. Percentage of trees in bud varied significantly in the third month after transplanting when about 90% of the rooted cuttings and large micropropagated plants had formed flower buds while only one multiple-planted seedling developed a bud. Overall, the clonally propagated plants were more vigorous and earlier bearing than were the seedling plants. There is good potential for adoption of clonal propagation when production becomes efficient enough to compete in price with the current practice of over planting and thinning.

Papaya (Carica papaya L.) is a polygamous species with all three sex forms: staminate (male), hermaphrodite, and pistillate (female). Sex is determined by a single gene with three alleles: M, male; Mh, hermaphrodite; and m, female (Hofmeyr, 1938; Storey, 1938, 1941, 1953). Storey (1939, 1953) reported crossing experiments that showed every combination of dominant alleles, M1M1, M1M2, and M2M2, will segregate one female to one hermaphrodite among seed obtained from different types of crosses. Hermaphrodite papaya trees are preferred for commercial production in Hawaii. Female papaya trees are not grown commercially as a result of their variably sized fruit and reduced seed content that leaves a large air space in the seed cavity as a result of unreliable cross-pollination. In addition, the round shape of the female fruit requires greater container volume for shipping than does the more slender pyriform-shaped fruit of the hermaphrodite trees. Hermaphrodite papaya orchards are generally established by planting five to ten selfed hermaphrodite seeds in a single hole. In this system, seedlings are grown for 3 to 4 months before they are thinned to remove the female plants, leaving a single hermaphrodite tree. This system of over-planting followed a few months later by thinning to a single tree is not only wasteful of seed, water, and fertilizer, but also intra-plant competition for sunlight results in more spindly plant growth and later bearing than when plants are grown with wider spacing. The cost for establishing orchards might be reduced and earlier fruit harvests might be achieved if there were a way to solve the sex segregation problem and establish orchards with hermaphrodite plants from the start. Clonal propagation from hermaphrodite plants is one way to acquire plant propagules of known sex.

Clonal propagation of papaya would not only solve the problem of providing plants of known sex, the plants would also be true to genotype. Traditionally, Hawaii’s papaya farmers have started orchards with farmer saved seed from selfed hermaphrodite inbred lines. Over the past few years, however, the predominant cultivar has been a transgenic F1 hybrid, 'Rainbow'. Selfed seed from 'Rainbow' segregates for numerous characters, including fruit color and quality and resistance to several diseases including papaya ringspot virus (PRSV), thus farmers must plant hybrid seed and there is no commercial supply. To avoid this problem, clonal propagation might be used to start a new crop. Clonal propagation could be through rooted cuttings taken from field- or greenhouse-grown trees or from shoots clonally multiplied in a laboratory tissue culture system, i.e., micropropagation.

Allan (1964, 1993) may have been the first to report a protocol for papaya cloning by rooting lateral branches of mature trees. Reuveni and Shlesinger (1990) increased the production of clonal propagules by using gibberellic acid (GA3) and benzylaminopurine (BA) spray treatments to increase the emergence of lateral branches from decapitated, greenhouse-grown stock plants. A combined treatment of 2.2 mM BA and 2.6 mM GA3, applied in lanolin paste to each nodal bud, or three spray treatments of 2.2 mM BA and 0.3 mM GA3 were used to stimulate multiple shoot growth. The emerged thin branches, 8 to 12 mm in diameter, were dipped in talcum powder containing 10 g L−1 benomyl and 49 mM indolebutyric acid (IBA) (potassium salt), placed in an aerated potting medium, and rooted with bottom heat (30°C) and intermittent mist. A total of 85% to 100% of the cuttings rooted after 21 d.

Papaya micropropagation was first reported by Litz and Conover (1977) and subsequently expanded (Chan and Teo, 2002; Drew, 1988; Drew et al., 1993; Drew and Vogler, 1993; Reuveni et al., 1990; and Yu et al., 2000). Field trials with clonally propagated papaya trees showed that they bore fruit earlier and lower on the trunk than did over-planted and thinned seedlings. In a trial in Queensland, Australia, flowering height was reduced from 1.5 to 2.0 m for typical thinned seedlings to 0.3 m to 0.4 m for micropropagated plants (Drew, 1988). In another experiment, height of first flowers of micropropagated females derived from adult tissues were compared to single seedling females planted in different seasons (Drew and Vogler, 1993). Micropropagated plants and seedlings planted in summer (February) bore flowers at significantly different heights, 43 and 56 cm, respectively (P = 0.01). Micropropagated plants and seedlings planted in late spring (November) bore flowers at even more disparate and significantly different heights, 32 and 79 cm, respectively (P = 0.01). In a trial conducted in Malaysia, three hermaphrodite
seedling selections developed their first flowers at 98 to 128 cm while micropropagated plants from these lines developed their first flowers at 80 to 82 cm (Chan and Teo, 2002).

Harvesting is one of the more costly operations in papaya production, especially for tall trees. Harvesting of fruit higher than a person's reach, about 183 cm, adds significant additional costs. Lower bearing trees help on harvest costs for a few months.

Because farmers need orchards with plants of known sex and genotype, there was a need for a study comparing tree growth and development of multiple-plant seedlings that are later thinned versus those established from clonally propagated rooted cuttings or micropropagated plants. In this, the first of two reports, we present results of establishing clonal propagules and field trials evaluating the growth and development of seedlings, rooted cuttings, and micropropagated plants in three locations over two years.

Materials and Methods

Plant material

All experiments were conducted with PRSV-resistant commercial papaya hybrid cultivar ‘Rainbow’. ‘Rainbow’ papaya seeds were gifts of Richard Manshardt, University of Hawaii.

Field sites

Keaau. The first established and most extensively sampled test was at Keaau on the island of Hawaii. The test was installed on 30 Apr. 1998 in Papai, an extremely stony soil (USDA, 1972, 1973) within a 750-year-old to 1500-year-old lava flow (USGS, 1996) at an elevation of 60 m. The average daily temperature was 28 °C in summer and 23 °C in winter with a soil temperature range of 22 to 23 °C. Rainfall at this site averages 381 cm annually (USDA, 1973). No additional irrigation is provided. The 30-year (1932 to 1975) solar radiation average from weather stations established at multiple-plant seedlings that are later thinned versus those established from clonally propagated rooted cuttings or micropropagated plants. In this, the first of two reports, we present results of establishing clonal propagules and field trials evaluating the growth and development of seedlings, rooted cuttings, and micropropagated plants in three locations over two years.

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rooted CTs were acclimatized for the three field tests.

**Seedlings**

About 5500 seedlings (SDs) were germinated for the three field tests including border rows. In total, 1800 SDs were required for the multiple-planted, thinned treatment (SD5); 600 SDs were tested for sex expression for the single-seeding (SD1) treatment; and 3000 SD5s were used to establish double-border rows surrounding each test.

Seeds were germinated in 2.5 × 2.5 × 3 cm peat pots filled with a peat based potting mixture. Pots were irrigated twice a day with overhead sprinklers. After germination, SDs were fertilized with (14N–14P2O5–14K2O) timed release granules. SDs for the Keaau test were germinated and grown in the greenhouse to 3 months of age when they reached 15 to 20 cm tall and 3 to 4 mm in diameter. The Helemano and Mokuleia SDs were germinated in an outdoor nursery at Dole Co., Haleiwa, Hawaii, to 7 to 8 weeks of age when they reached 15 to 30 cm tall and 3 to 4 mm in diameter. Seedlings used for PCR testing were randomly selected from among the two batches.

**PCR for sex determination**

DNA was extracted from two leaves of seedlings macerated in CTAB extraction buffer (Dellaporta et al., 1983; Saghai-Marooif et al., 1994) as previously described (Fitch et al., 1992). PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). The primers, developed from RAPD PCR was performed using primers T1 (20-mer) and T12 (21-mer) (Deputy et al., 2002). 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types was significantly higher than the SD5s (Table 3). Nearly all of the plants were in bud by the fifth month. Height of the first bud differed between the treatments with the SD5s flowering highest at 89.9 cm (1 plant) in the third month after transplant followed by SD1, MP, and CT plants (Table 4). By the fifth month, the SD5s were still significantly the highest bearing followed by SD1, MP, and MP plants which were all significantly higher bearing than CTs (53.9 cm). The fruit height remained the same for the next 2 months of measurement. Bud height averages varied from month to month as increasing numbers of trees developed buds and, occasionally, the earliest buds abscised. In the fifth month after transplanting, nearly all of the plants had formed buds so that bud height data for months 4 to 7 were nearly identical.

The percentage of trees with harvest height exceeding 183 cm (harvesting devices required) differed among the plant types (Table 4). At 18 months after transplanting, about one-third (33.8%) of all SD5 trees had to be harvested with poles, a significantly higher percentage compared to CT (0%), MP (1.3%) and MP (7.5%) plants. Harvesting poles were required for 15.6% of the SD1 plants, not significantly different from SD5 nor the clonally propagated plants.

Helemano height and diameter measurements were taken four times in the growth cycle (Table 5). Despite the small number of data points, significant differences were observed between the taller SD5 and clonally propagated plants in each sampling. There were no differences in diameter in the third to sixth months but micropropagated plants were thicker in the seventh month.

Bud heights 3 months after transplanting in Helemano were higher for SD5 plants (55.7% budded) than for MPs (45% budded) and CTs (35% budded), but the data were not significantly different (Table 6). CTs continued to have a significantly lower percentage of trees with buds in the fourth month of growth. At 7 months of growth, all plants had formed flower buds, and SD5s were significantly higher bearing than MPs and CTs that were equal in bud height. Later in the growth cycle, more SD5 plants required harvesting devices compared to the clonally propagated plants (Table 7). An average of 91% of the SD5 exceeded 183 cm compared to 44% of the MPs and 32% of the CTs.

Plant types in Mokuleia differed significantly (P<0.05) in height and but not diameter in the first month of growth (Table 8), a reflection of initial planting size differences. SD5 plants were significantly taller than all other plant types for the duration of the experiment. SD1 plants were taller than clonally propagated plants in the first 10 months of growth except for the third month when there was no differ-

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**Fig. 1.** Contrasting methods for production of papaya. (A) Multiple-planted, thinned seedlings in a commercial field on Oahu. (B) Rooted cuttings and (C) multiple-planted, thinned seedlings 7 months after planting in lava in Keaau. The trees in B and C were transplanted at the same time.
Table 2. Monthly growth of cloned or seedling papaya plants at the Keaau site.

| Plant type | Month (date) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------|--------------|---|---|---|---|---|---|---|---|---|
| CT         | (May 1998)   | 19.6 b | 40.6 b | 57.1 b | 80.2 b | 100.3 b | 116.7 a | 133.3 a | 136.7 b | 147.6 b |
| MP         | (June 1998)  | 30.4 a | 53.4 a | 73.2 a | 96.6 ab | 117.5 ab | 133.5 a | 149.2 a | 158.0 ab | 163.0 ab |
| MPm        | (July 1998)  | 19.3 b | 39.0 b | 61.0 ab | 85.7 b | 111.5 b | 124.2a | 139.9 a | 153.4 ab | 155.2 ab |
| SD1        | (Aug. 1998)  | 11.5 c | 36.3 b | 58.9 ab | 84.0 b | 108.1 ab | 125.9 a | 143.9 a | 157.7 ab | 160.3 ab |
| SD5        | (Sept. 1998) | 18.6 b | 42.4 b | 62.9 ab | 105.7 a | 129.5 a | 143.1 a | 160.6 a | 173.0 a | 180.0 a |

Tree height (cm)

CT = cutting plants, MP = large micropropagated plants, MPm = small micropropagated plants, SD1 = single hermaphrodite seedlings, SD5 = multiple-planted then thinned seedlings.

Table 3. Percentage of cloned and seedling papaya plants in bud at Keaau.

| Plant type | Month (date) | 2 | 3 | 4 | 5 | 6 |
|------------|--------------|---|---|---|---|---|
| CT         | (June 1998)  | 0 | 88.8 a | 98.8 a | 100 a | 100 a |
| MP         | (July 1998)  | 0 | 92.5 a | 100 a | 100 a | 100 a |
| MPm        | (Aug. 1998)  | 0 | 54.3 b | 97.6 a | 100 a | 100 a |
| SD1        | (Sept. 1998) | 0 | 27.0 c | 83.4 a | 100 a | 100 a |
| SD5        | (Oct. 1998)  | 0 | <1.0 d 60.2 b 95.0 a | 100 a | 100 a | 100 a |

Tree diameter (cm)

CT = cutting plants, MP = large micropropagated plants, MPm = small micropropagated plants, SD1 = single hermaphrodite seedlings, SD5 = multiple-planted then thinned seedlings.

Table 4. First bud or fruit height of cloned or seedling papaya plants at Keaau.

| Plant type | Month (date) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------|--------------|---|---|---|---|---|---|---|---|---|
| CT         | (May 1998)   | 49.4 d | 54.0 c | 53.9 d | 52.3 c | 54.2 c | 54.2 c | 54.2 c | 54.2 c | 54.2 c |
| MP         | (June 1998)  | 66.9 bc | 72.0 b | 70.9 bc | 71.4 b | 69.8 b | 69.8 b | 69.8 b | 69.8 b | 69.8 b |
| MPm        | (July 1998)  | 59.6 e | 68.9 b | 66.7 e | 67.4 b | 65.5 b | 65.5 b | 65.5 b | 65.5 b | 65.5 b |
| SD1        | (Aug. 1998)  | 73.7 b | 76.4 b | 77.2 b | 75.8 b | 71.6 b | 71.6 b | 71.6 b | 71.6 b | 71.6 b |
| SD5        | (Sept. 1998) | 89.9 a | 106.3 a | 103.8 a | 106.8 a | 104.1 a | 104.1 a | 104.1 a | 104.1 a | 104.1 a |

Discussion

Trees grown from seed developed differently from the micropropagated or cutting-derived plants by being taller, bearing flowers and fruit higher on the stem, and flowering at an intermediate time. As in most species, this behavior can be explained by the known effect of the juvenility factor which persists longer in seedlings. These data however differ from those of Drew and Vogler (1993) who reported that clones micropropagated from juvenile tissue did not differ consistently from seedlings in height and node number of first flower and in growth parameters. These authors reported that only plants micropropagated from adult tissue had a reduced juvenile phase indicated by increased circumference, lower height of first flower, reduced time to harvest, and higher fruit numbers per measured stem. Different ecological conditions and different plant materials (dioecious lines in Australia) can possibly play a role in explaining the differences. The Mokuleia data support some of the Australian observations because, while 90% of the plants were micropropagated from juvenile tissue, they did not differ from single-planted seedlings in diameter, were shorter in height only in contrast, an average of 51% of thinned seedlings, but <2% of all other plants in Mokuleia, required harvesting devices 10 months after planting. However, 4 months later, >85% of all trees had to be harvested with ladders and there was no significant difference among the plant types.

One tree in four replication blocks.

Columns at the right = percentage of trees requiring harvesting devices.

<sup>CT</sup> = cutting plants, <sup>MP</sup> = large micropropagated plants, <sup>MPm</sup> = small micropropagated plants, <sup>SD1</sup> = single hermaphrodite seedlings, <sup>SD5</sup> = multiple-planted then thinned seedlings.

<sup>Mean values within a column followed by different letters indicate significant differences among treatments</sup> (P < 0.05) according to ANOVA (LSD).

<sup>Data from females and hermaphrodites. Females were removed if sex expression could be determined.</sup>

<sup>Fruit height for SD5 plants was significantly shorter than those of CTs and MPs (Table 10). At the first harvest after 8 months of growth, fruit height of 7.5% of the SD5 trees exceeded 183 cm, the maximum height for harvesting without additional devices like poles, ladders, scissorlifts, or forklifts. Ten months after transplanting, >50% of the SD5 trees were harvested with ladders compared to <2% of the other plant types. In the last data collection, 14 months after transplanting, the fruit of all plant types were harvested with ladders, but fruit height for SD5 plants was significantly higher than all other plants. Fruit height of MP plants was significantly lower than seedlings.</sup>

<sup>Variations in monthly height data were a result of the changing number of mature plants and abscission of some early buds. The first bud or fruit on SD5 plants was significantly higher compared to all other plant types from the third month of growth (Table 10). At the first harvest after 8 months of growth, fruit height of 7.5% of the SD5 trees exceeded 183 cm, the maximum height for harvesting without additional devices like poles, ladders, scissorlifts, or forklifts. Ten months after transplanting, >50% of the SD5 trees were harvested with ladders compared to <2% of the other plant types. In the last data collection, 14 months after transplanting, the fruit of all plant types were harvested with ladders, but fruit height for SD5 plants was significantly higher than all other plants. Fruit height of MP plants was significantly lower than seedlings. Different large differences were observed in fruiting height of the plant types at the three test sites. At 18 months after transplanting, >93% of all cloned propagated trees in Keaau and significantly fewer, 66%, of the thinned seedlings were harvested without picking devices. In contrast, an average of 51% of thinned seedlings, but <2% of all other plants in Mokuleia, required harvesting devices 10 months after planting. However, 4 months later, >85% of all trees had to be harvested with ladders and there was no significant difference among the plant types.</sup>

<sup>Discussion</sup>

Trees grown from seed developed differently from the micropropagated or cutting-derived plants by being taller, bearing flowers and fruit higher on the stem, and flowering at an intermediate time. As in most species, this behavior can be explained by the known effect of the juvenility factor which persists longer in seedlings. These data however differ from those of Drew and Vogler (1993) who reported that clones micropropagated from juvenile tissue did not differ consistently from seedlings in height and node number of first flower and in growth parameters. These authors reported that only plants micropropagated from adult tissue had a reduced juvenile phase indicated by increased circumference, lower height of first flower, reduced time to harvest, and higher fruit numbers per measured stem. Different ecological conditions and different plant materials (dioecious lines in Australia) can possibly play a role in explaining the differences. The Mokuleia data support some of the Australian observations because, while 90% of the plants were micropropagated from juvenile tissue, they did not differ from single-planted seedlings in diameter, were shorter in height only in contrast, an average of 51% of thinned seedlings, but <2% of all other plants in Mokuleia, required harvesting devices 10 months after planting. However, 4 months later, >85% of all trees had to be harvested with ladders and there was no significant difference among the plant types.
Table 5. Monthly growth of cloned or seedling papaya plants at the Helemano site.

| Plant type | Month (date) after transplanting cloned or seedling plants of papaya | 2 (Dec. 1998) | 3 (Jan. 1999) | 4 (Feb. 1999) | 6 (April 1999) | 7 (May 1999) |
|------------|---------------------------------------------------------------|--------------|--------------|--------------|--------------|-------------|
| Tree height (cm) | | | | | | |
| CT | ND | 63.3 b | 77.9 b | 128.3 b | 153.7 b |
| MP | ND | 66.3 b | 86.9 b | 130.4 b | 162.6 b |
| SD5 | ND | 88.6 a | 112.9 a | 172.9 a | 205.1 a |
| Tree diameter (cm) | | | | | | |
| CT | ND | 4.7 a | 5.5 a | 10.3 a | 12.1 b |
| MP | ND | 4.6 a | 5.4 a | 10.3 a | 16.6 a |
| SD5 | ND | 4.7 a | 5.8 a | 9.8 a | 11.9 b |

*CT = cuttings, MP = micropropagated plants, SD5 = thinned seedlings.

ND = no data were collected.

Mean values within a column followed by different letters indicate significant differences among treatments \( P < 0.05 \) according to ANOVA (LSD).

Table 6. Percentage of cloned or seedling papaya trees in bud at Helemano.

| Plant type | Month (date) after transplanting cloned or seedling papaya plants | 1 (Nov. 1998) | 2 (Dec. 1998) | 3 (Jan. 1999) | 4 (Feb. 1999) | 6 (April 1999) | 7 (May 1999) |
|------------|---------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|-------------|
| CT | 0 | ND | 66.2 a | 76.0 b | 80.9 b | 78.5 b |
| MP | 0 | ND | 70.2 a | 71.3 b | 80.4 b | 78.1 b |
| SD5 | 0 | ND | 86.8 a | 104.6 a | 114.5 a | 115.4 a |

*CT = cuttings, MP = micropropagated plants, SD5 = thinned seedlings.

ND = no data were collected.

Mean values within a column followed by different letters indicate significant differences among treatments \( P < 0.05 \) according to ANOVA (LSD).

*Females were removed after data were collected.

Table 7. First bud or fruit height of cloned or seedling papaya plants at Helemano.

| Plant type | Months and dates after transplanting cloned or seedling papaya plants | 1 (Nov. 1998) | 2 (Dec. 1998) | 3 (Jan. 1999) | 4 (Feb. 1999) | 6 (April 1999) | 7 (May 1999) |
|------------|---------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|-------------|
| Trees with fruit columns | >183 cm (%) | | | | | | |
| CT | 0 | ND | 66.2 a | 76.0 b | 80.9 b | 78.5 b |
| MP | 0 | ND | 70.2 a | 71.3 b | 80.4 b | 78.1 b |
| SD5 | 0 | ND | 86.8 a | 104.6 a | 114.5 a | 115.4 a |

*CT = cuttings, MP = micropropagated plants, SD5 = thinned seedlings.

ND = no data were collected.

Mean values within a column followed by different letters indicate significant differences among treatments \( P < 0.05 \) according to ANOVA (LSD).

Table 8. Monthly growth of cloned or seedling papaya plants at the Mokuleia site.

| Plant type | Month (date) after transplanting cloned or seedling papaya plants | 1 (April 1999) | 2 (May 1999) | 3 (June 1999) | 4 (July 1999) | 5 (Aug. 1999) | 6 (Sept. 1999) | 7 (Oct. 1999) | 8 (Nov. 1999) | 9 (Dec. 2000) | 10 (Jan. 2001) | 11 (Feb. 2001) | 12 (March 2001) | 13 (April 2001) |
|------------|---------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Height (cm) | | | | | | | | | | | | | | |
| CT | 27.9 c | 55.2c | 97.5 b | 126.5 c | 167.7 c | 195.1 b | 230.1 bc | 265.6 c | 275.8 bc | 296.6 bc | 308.5 b | 319.7 b | 340.6 b | 349.0 bc |
| MP | 33.2 c | 57.1 bc | 94.7 b | 121.7 c | 162.8 c | 187.7 c | 222.6 c | 251.1 c | 269.5 c | 287.2 c | 301.1 b | 313.1 b | 330.2 b | 333.6 c |
| SD1 | 39.7 b | 63.7 b | 104.6 b | 143.3 b | 187.9 b | 210.6 b | 246.8 b | 287.3 b | 293.8 b | 312.4 b | 325.5 ab | 327.3 b | 354.4 b | 360.1 b |
| SD5 | 46.6 a | 80.6 a | 147.4 a | 180.4 a | 222.1 a | 247.6 a | 286.4 a | 316.4 a | 354.5 a | 385.1 a | 397.1 a | 404.1 a | 404.6 a |
| Diameter (cm) | | | | | | | | | | | | | | |
| CT | 1.4 a | 3.6 a | 7.1 a | 10.3 a | 14.5 a | 17.7 a | 18.3 a | 20.2 a | 20.3 a | 20.6 a | 20.6 a | 21.0 a | 20.9 ab | 23.2a |
| MP | 1.4 a | 2.8 a | 6.6 a | 9.5 a | 13.6 a | 16.3 a | 17.8 a | 19.5 a | 20.1 a | 20.3 a | 20.4 a | 20.2 a | 20.8 ab | 20.2 a |
| SD1 | 1.5 a | 3.6 a | 7.3 a | 10.4 a | 14.5 a | 17.8 a | 18.4 a | 20.1 a | 20.4 a | 20.6 a | 21.0 a | 21.9 a | 20.8 ab | 21.0 a |
| SD5 | 1.3 a | 3.1 a | 5.8 a | 9.3 a | 14.0 a | 17.0 a | 18.5 a | 20.2 a | 19.5 a | 21.2 a | 21.4 a | 21.1 a | 22.8 a | 21.4 a |

*CT = cuttings, MP = micropropagated plants, SD1 = single hermaphrodite seedlings, SD5 = thinned seedlings.

Mean values within a column followed by different letters indicate significant differences among treatments \( P < 0.05 \) according to ANOVA (LSD).

Table 9. Percentage of cloned or seedling papaya plants in bud at Mokuleia.

| Plant type | Months and dates after transplanting cloned or seedling papaya plants | 1 (May 1999) | 2 (June 1999) | 3 (July 1999) |
|------------|---------------------------------------------------------------|--------------|--------------|--------------|
| CT | 12.8 a | 81.0 a | 100 a |
| MP | 5.5 a | 78.9 a | 100 a |
| SD1 | 13.0 a | 76.4 a | 100 a |
| SD5 | 3.6 a | 74.1 a | 100 a |

*CT = cuttings, MP = micropropagated plants, SD1 = single hermaphrodite seedlings, SD5 = thinned seedlings.

Mean values within a column followed by different letters indicate significant differences among treatments \( P < 0.05 \) according to ANOVA (LSD).

*Data for females and hermaphrodites. Females were removed if sex expression could be determined.
5 of the 14 months of growth and had lower bud or fruit height in only the earliest months of growth (6 of the 14 months). Mokuleia had high levels of solar irradiation coupled with the highest average daily temperatures. Juvenility was pronounced in the first 3 months of growth in Keaau micropropagated plants all of which were derived from seedling tissues. Both large and small micropropagated plants were significantly shorter than single seedlings in the first month of growth and the percentage of micropropagated trees in bud was significantly higher than single seedlings when buds were first observed 3 months after transplant.

Before beginning this project, there was some concern that micropropagated plants might not develop a sufficiently sturdy root system since they would not have tap roots, or that the trees might senesce earlier, or the micropropagated plants might not be as productive as seedlings. None of these problems were observed and our results corroborate those of earlier studies by Allan (1964, 1993), Chan and Teo (2002), Drew (1988), and Drew and Vogler (1993) that showed earlier and lower flowering and fruiting of clonally propagated papayas compared to thinned seedlings. Allan in South Africa and Drew in Australia studied dioecious papayas that are generally grown in temperate regions where cooler winters result in serious fruit losses as a result of carpelddy and male sterility. In those more temperate zone systems, maximum production is obtained by surrounding female trees with pollen bearing male trees. Our micropropagated plants fruited at 54±78 cm, comparable to micropropagated hermaphrodite Hawaiian type papayas in Malaysia at 80±8 cm (Chan and Teo, 2002), which was higher than with the dioecious Australian trees at 30 to 40 cm (Drew, 1988). Thinned seedlings in Hawaii fruited at 104 to 137 cm versus 105±38 cm for similar genotypes in Malaysia and 150 to 200 cm for dioecious genotypes in Australia. Australian papayas showed the greatest differences in fruiting height between micropropagated plants and thinned seedlings. Neither they nor we observed significant differences in tree diameter.

Zhou and coworkers (2000) studied the physiological effects of source (photosynthetic leaf, assimilate) on sink (fruit or fruit set) in papaya and reported that if the size of assimilate capacity were larger than the sink demand, then new flowers and fruit would continue to be set. While node number counts predict the transition time from juvenile to adult papaya for any given cultivar (Nakasonke and Storey, 1955), those data may be another manifestation of the source or sink concept in flower bud initiation. Apparently the clonally propagated plants in Keaau had avoided juvenility to form flowers earlier than both single-plant and thinned seedlings. More rapid growth in Mokuleia and Helemano, most likely as a result of higher temperature, solar radiation, and fertility, may have minimized the juvenile period in seedlings since they flowered at about the same time and rate as the clonally propagated plants. While there was no apparent advantage to eliminate crowding on seedlings in terms of the timing and percentage of all plants to form flower buds, flowering height was highest in thinned seedlings at all three locations. Thus, while clonally propagated plants flowered earlier than seedlings in Keaau, the major effect of clonally propagated plants at all three locations was the lower flowering height. The shorter clonally propagated trees being easier to harvest for a few months in the cropping cycle, help to reduce harvest costs and consequently benefits growers.

Variation in plant phenotype, a problem associated with in vitro propagation, was not observed in this study that included 350 micropropagated trees, >80% of which originated from nine in vitro-germinated seedlings. Drew and Vogler (1993) described four dwarf off-type plants (<1% of population) that originated from a single bud explant. Since the Australian off-types were traced to a single bud from the selected mature tree, we believe that the variation originated from the tree rather than from tissue culture manipulations. In our experience with hybrid papaya trees, we observed at least five seedlings among several thousand that had thicker than normal leaves, petioles and stems, larger than normal flowers and leaf scars, and were nearly sterile. We believed they were tetraploids but did not check ploidy. At least two of the sterile trees developed fertile, normal-looking lateral branches that we rooted as cuttings and that produced normal-looking fruit in highly productive fruit columns.

The major bottlenecks to clonal propagation are 1) high cost of production as a result of low survival rates of plants grown as rooted cuttings or micropropagated plants and 2) timed output of propagules to enable farmers to plant when fields are prepared. In reference to the first bottleneck, our experience in scaling up production showed that problems with contamination prevented high throughput (Fitch et al., 2003a, 2003b). Therefore, we have revised protocols in which losses as a result of contamination are minimized, for example, decreasing or removing sugar from some of the growth media and incorporating bacteriocides in the media. To address the second bottleneck we note that plants can be maintained for a few months in the greenhouse or nursery the same as seedlings.

The vegetative cutting technology initiated from lateral branches is limited by the number of branches that can be harvested from selected trees. While this method may be adequate for the small numbers of plants required for the backyard gardener or small grower, micropropagation has the greater potential for providing enough plants for the 1500 ha under cultivation in Hawaii.

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