

Challenges in breeding and selecting *Passiflora edulis* f. *flavicarpa* × *P. incarnata* hybrids

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Abstract

Passiflora is comprised of many species, with *P. edulis* being the prominent commercial species. Another species that grows in more temperate regions is *P. incarnata*. Breeding to create interspecific hybrids with these two species has been done before but without longstanding success. Controlled crosses of *P. edulis* f. *flavicarpa* × *P. incarnata* were made to generate baseline data on interspecific passionfruit hybrids that can survive subtropical and temperate winters. The number of fruits collected per individual hybrid selection ranged from 4 to 52. Some fruits produced no seeds whereas others averaged more than 25 seeds per fruit. Pulp weight ranged from 0 to 10 g, with two individuals above 10 g. Many vines had poor pulp percentage (< 25%). A few were over 30% and two individuals were over 40%. The average width of fruit ranged between 32 and 46 mm. The overall shapes were similar among fruits. Vines with *P. incarnata* from Illinois (USA) as a parent produced fewer seeds, lower total fruit weight, shorter height, smaller width, lower fruit density, lighter pulp weight and pulp percentage. The vines with *P. incarnata* from Oklahoma (USA) as a parent made more seeds, longer height, and a more elongated shape than the other two pollen parents. Mississippi-based *P. incarnata* vines had the greatest average total fruit weight, hull weight, fruit width, and fruit density. Based on the results of this study, there is reason to be cautiously optimistic about interspecific hybrid *Passiflora* involving *P. incarnata*. Creating more generations with backcrossing to *P. edulis* is the next logical step in the process.

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Introduction

Passionfruit (*Passiflora* sp.) is primarily a tropical fruit, originating in South America with *Passiflora edulis* being the most important commercial species^[1,2]. However, other species within *Passiflora* exist in subtropical and temperate areas. Some of these species produce fruit that may potentially be used in breeding for more cold hardy passionfruit vines. One of the most interesting of these species is *P. incarnata*, also called maypop. It is native to North America, especially the southeastern US^[3–6]. It is most well-known for its pharmaceutical properties and is widely used as an anti-spasmodic and sedative^[7,8]. This diploid, herbaceous, perennial vine also can produce a sizable, edible fruit. In areas of the US where the vine is native, it is often considered a weed, as it will colonize disturbed areas within crop land or along fence rows^[9]. Wild fruit of *P. incarnata* is highly variable in size and fruit quality and little effort has been made in selecting superior individuals for breeding. The distribution of the vine is broad, encompassing many different climates, from cold and wet (PA, NY, USA) to hot and dry (TX, USA)^[6]. Evaluation of vines from across the spectrum of locations would enhance breeding efforts, but only limited work in this area has been performed.

P. incarnata is also self-incompatible which further inhibits breeding progress^[10]. Interspecific hybridization could potentially help with compatibility issues, although previous attempts have not proved successful. Knight^[11–13] tried for many years to cross *P. edulis* and *P. incarnata* for improved fruit quality, but a successful fruit-bearing cultivar was never

released, only a colchicine-induced tetraploid ornamental ('Byron Beauty')^[14].

Passionfruit is gaining in popularity around the globe^[15], but *P. edulis* cannot be grown everywhere successfully as it is tropical in nature and will not survive cold winter temperatures. Therefore, the purpose of this work is to develop baseline data on interspecific passionfruit hybrids involving *P. incarnata* that can survive subtropical and temperate winters while prospering in areas that have relatively long, hot summers.

Materials and methods

The study began in 2021 and fruit was collected in 2022 at the Mississippi State University South Mississippi Branch Experiment Station in Poplarville, Mississippi, USA (lat. 30°85'36" N, long. 89°49'94" W, elevation 97 m, USDA hardiness zone 8b). *Passiflora incarnata* parental vines were from Villa Park, Illinois (IL), Richton, Mississippi (MS), and Guthrie, Oklahoma (OK). *P. incarnata* were obtained from wild plants, either *via* seed or root cutting. Controlled crosses (*P. edulis* f. *flavicarpa* × *P. incarnata*, Fig. 1) that resulted in the seedlings tested in this study were performed under a high tunnel structure where *P. edulis* f. *flavicarpa* was planted in the ground. Controlled hand pollinations were done once a flower had opened, generally between 11:00 am and 1:00 pm. An anther was removed, and pollen was rubbed on the stigma. The pollinated flower was covered by a mesh bag. Evidence of positive fertilization was observed usually within 48 h, but final determination of success was not made until fruit fully developed. Successfully developed fruit



Fig. 1 Representative flower types used in interspecific crosses to develop more cold hardy fruiting passionfruit vines. *Passiflora incarnata* flower (left), *P. edulis* f. *flavicarpa* flower (middle), and resulting hybrid flower (right).

were collected when ripe and seeds were extracted. Due to limitations imposed by the COVID-19 pandemic, seeds were stored in a cool, dry environment for up to 1 year before sowing. Collected seeds were submerged in hot water and allowed to sit for 24 h prior to sowing under intermittent mist in a greenhouse. Upon emergence, all seedlings remained in the greenhouse and/or fully enclosed screened high tunnel but were removed from intermittent mist once vining habit and tendril growth started. At that stage they were then re-potted into 11.4-L pots (3-gal) pots and hand watered as needed. The vines were eventually put onto a gravel-covered nursery pad in rows with pots spaced 1.83 m (6 ft) apart within the row and 2.44 m (8 ft) between rows after chance of frost had passed and were drip irrigated. All vines were fertilized with 16 g 13-13-13 (N-P-K, Magic Carpet, Agri-AFC LLC, Decatur, AL, USA) after installation on gravel pad. Vines were allowed to attach to wire fencing material up to 1.52 m (5 ft) tall. In total there were 35 vines. Once on the gravel pad, pollinations were allowed to be made naturally *via* insect visitation. Eastern Carpenter Bees (*Xylocopa virginica*) are common in Mississippi and around these vines. Therefore, no hand pollination was done. Fruits were collected regularly once they naturally abscised from the vine from 5 July to 7 Sept. 2022. All fruit were processed immediately where they were measured for length and width with a Mitutoyo Absolute Digimatic (Mitutoyo Corp., Kawasaki, Japan). Fruit were weighed both as entire fruit and once pulp was removed. Fruit density was determined by the formula: $F_d = W/(H \times D)$, where W = fruit weight (g), H = fruit height (mm), and D = fruit width (mm). Fruit shape was calculated as H/D as described in Md Nor et al.^[16]. Fruits were rated based on feel on a scale of 1 to 5, with 1 = light, empty fruit to 5 = heavy, full fruit. A visual rating of pulp content was also done after the fruits were cut in half, also on the same 1 to 5 scale.

Data of fruit measurements were analyzed by JMP (version 12; SAS Institute, Cary, NC, USA) using a one-way analysis of variance and means were compared with standard error of the mean or Tukey's honestly significant difference (HSD) at the 0.05 level where appropriate. Pearson product-moment correlations were performed in the multivariate procedure.

Results

In total there were 35 seedling vines. Some of these had already been through one round of selection in the previous year (2021). Out of these 35, 11 were kept in 2022 as advanced

selections (Table 1). The number of fruits collected per individual ranged from 4 (22R504) to 52 (20R104 and 21R308). The former had *P. incarnata* IL as a parent and the latter two had *P. incarnata* OK. Fruits were rated based on feel to gauge, non-destructively, the mass of the fruit. Most average ratings were between 3 and 4 but ranged from 1.59 (22R507) to 4.58 (20R314). After the feel rating, another the visual rating of the pulp was performed, also on a 1-5 scale. The purpose was to see how closely the ratings were aligned. The lowest ranked individuals were 22R502 and 22R504 (1.00) and the highest was 20R314 (4.50). These two ranking were significantly correlated ($p < 0.0001$) at 0.6586 (Table 2). In 25 out of 35 cases the feel rating was higher than the visual.

Seed number was counted for each fruit and then averaged over all collected fruits for each individual vine. Some fruits produced no seeds (22R403 and 22R504) whereas others had more than 25 seeds per fruit (20R305 and 21R319) (Table 1). The presence of many seeds was an indicator of significant pulp presence. As would be expected with any segregating population only one generation from wild material, average total weight of the fruit varied greatly among the individual vines. Some were small, close to 10 g (22R507) and some were much larger, over 30 g (20R305). Of course, there are different components encompassing the total weight, namely hull weight and pulp weight (including seeds). Hulls of *P. incarnata* are thinner than those of *P. edulis* and that difference in thickness can affect postharvest shelf life. In general, hull weights ranged between 10 to 20 g, with a few less than 10 g and only one at 20 g (Table 1). Pulp weight ranged from 0 to 10 g, with two individuals above 10 g (20R305 and 21R319), both of which have *P. incarnata* OK as a parent. Pulp % [(pulp weight/total weight) \times 100] was a strong indicator for advancing a selection. Many vines had poor pulp percentage (< 25%). A few were over 30% and two individuals were over 40% (20R114 and 21R319). Fruit density is a non-destructive measure of how full a fruit is of pulp while accounting for fruit size (g/cm²). Fruit densities were mostly < 1.00 but some were higher.

In terms of harvested fruit, shapes were almost exclusively longer than wide. Most were greater than 40 mm and many over 50 mm and only two were less than 40 mm (22R501 and 22R503). The average width of fruit ranged between 32 and 46 mm. The overall shape (height/width) were similar among all fruits with all being over 1.00 (round) and they were categorized as oblong spheroid^[16].

Table 1. Fruit related characteristics of interspecific hybrid Passiflora selections among both selections were kept for further evaluation and those that were eliminated from further analysis (not kept).

| Selection | Parentage | Fruit # | Keep | Feel ¹ | Visual ² | Seed # | Tot wt (g) | Hull wt (g) | HT (mm) | Width (mm) | Density (g/cm ³) ³ | Shape ⁴ | Pulp wt (g) | Pulp % ⁵ |
|-----------|--|---------|------|-------------------|---------------------|--------|------------|-------------|---------|------------|---|--------------------|-------------|---------------------|
| 20R104 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 52 | No | 2.38 | 1.98 | 5.67 | 16.37 | 12.47 | 55.40 | 41.43 | 0.69 | 1.34 | 2.20 | 11.59 |
| 20R114 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 37 | Yes | 3.78 | 4.27 | 21.91 | 22.61 | 12.60 | 56.97 | 39.10 | 0.99 | 1.46 | 9.57 | 40.15 |
| 20R305 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 22 | Yes | 4.45 | 4.36 | 25.58 | 32.17 | 19.11 | 59.16 | 44.59 | 1.18 | 1.33 | 10.36 | 33.21 |
| 20R309 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 14 | No | 3.93 | 3.14 | 9.71 | 24.89 | 15.17 | 56.38 | 42.30 | 0.98 | 1.33 | 3.17 | 12.19 |
| 20R314 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 26 | Yes | 4.58 | 4.50 | 22.74 | 29.54 | 17.03 | 54.49 | 42.39 | 1.21 | 1.29 | 9.52 | 33.25 |
| 20R426 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 21 | No | 3.29 | 3.55 | 11.27 | 18.56 | 14.22 | 47.57 | 35.71 | 1.01 | 1.35 | 5.01 | 20.81 |
| 21R203 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 24 | Yes | 3.29 | 3.33 | 14.56 | 18.94 | 11.98 | 55.52 | 40.65 | 0.82 | 1.37 | 5.26 | 27.74 |
| 21R206 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 51 | Yes | 3.24 | 3.22 | 10.38 | 17.38 | 11.18 | 49.02 | 33.08 | 0.99 | 1.49 | 5.32 | 25.90 |
| 21R207 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 27 | No | 2.30 | 2.00 | 6.96 | 12.03 | 9.11 | 50.83 | 34.39 | 0.66 | 1.49 | 2.68 | 20.13 |
| 21R215 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 20 | No | 3.95 | 3.85 | 13.47 | 21.47 | 14.40 | 57.14 | 38.95 | 0.94 | 1.47 | 5.47 | 25.75 |
| 21R217 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 33 | No | 2.82 | 2.82 | 6.20 | 15.78 | 11.74 | 53.01 | 38.08 | 0.77 | 1.40 | 2.50 | 14.36 |
| 21R218 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 33 | Yes | 3.06 | 3.39 | 18.33 | 18.14 | 9.67 | 52.91 | 37.65 | 0.87 | 1.41 | 6.47 | 32.59 |
| 21R221 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 31 | No | 2.68 | 3.00 | 15.56 | 18.97 | 13.15 | 56.73 | 40.18 | 0.82 | 1.41 | 5.50 | 28.06 |
| 21R301 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 41 | No | 3.05 | 3.17 | 15.50 | 17.93 | 12.19 | 52.28 | 37.83 | 0.87 | 1.39 | 7.39 | 34.79 |
| 21R303 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 41 | Yes | 3.95 | 3.66 | 17.13 | 23.12 | 14.62 | 54.90 | 40.66 | 0.99 | 1.35 | 6.86 | 28.74 |
| 21R304 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 43 | Yes | 3.09 | 3.30 | 14.67 | 18.21 | 11.72 | 54.10 | 37.50 | 0.86 | 1.45 | 6.06 | 28.37 |
| 21R308 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 52 | No | 2.27 | 2.44 | 7.59 | 15.03 | 10.06 | 53.58 | 38.94 | 0.69 | 1.38 | 3.14 | 19.50 |
| 21R317 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 6 | No | 2.33 | 1.33 | 2.00 | 20.48 | 20.00 | 54.17 | 44.91 | 0.83 | 1.20 | 0.85 | 2.35 |
| 21R319 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> OK | 41 | Yes | 3.41 | 4.07 | 26.71 | 22.28 | 10.27 | 56.57 | 38.96 | 0.98 | 1.45 | 10.99 | 46.97 |
| 22R306 | Frederick × <i>P. incarnata</i> IL | 30 | No | 2.43 | 2.37 | 9.07 | 14.40 | 10.28 | 48.43 | 39.87 | 0.74 | 1.22 | 3.90 | 26.25 |
| 22R307 | Frederick × <i>P. incarnata</i> IL | 25 | Yes | 3.36 | 3.88 | 19.57 | 18.62 | 11.78 | 52.80 | 39.13 | 0.89 | 1.35 | 6.87 | 36.87 |
| 22R401 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 16 | No | 3.13 | 2.19 | 6.92 | 25.03 | 15.35 | 52.80 | 46.63 | 0.94 | 1.14 | 3.38 | 17.33 |
| 22R402 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> MS | 35 | No | 3.26 | 2.31 | 7.42 | 20.66 | 13.90 | 53.15 | 41.97 | 0.90 | 1.27 | 3.72 | 19.77 |
| 22R403 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> MS | 5 | No | 3.00 | 1.40 | 0.00 | 18.51 | 13.00 | 47.19 | 36.52 | 1.04 | 1.31 | 0.00 | 0.00 |
| 22R404 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> MS | 12 | No | 3.75 | 3.50 | 12.00 | 21.39 | 13.09 | 50.33 | 37.40 | 1.10 | 1.35 | 6.14 | 26.96 |
| 22R405 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> MS | 20 | Yes | 3.85 | 3.35 | 14.64 | 25.77 | 17.31 | 55.36 | 43.77 | 1.04 | 1.27 | 7.55 | 29.30 |
| 22R406 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> MS | 20 | No | 3.45 | 2.40 | 10.75 | 21.57 | 15.12 | 49.35 | 41.22 | 1.05 | 1.20 | 5.93 | 24.84 |
| 22R501 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 16 | No | 3.75 | 3.25 | 11.20 | 23.00 | 15.22 | 48.57 | 40.47 | 1.14 | 1.20 | 5.58 | 27.77 |
| 22R502 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 23 | No | 1.61 | 1.13 | 1.05 | 11.73 | 11.10 | 39.04 | 34.34 | 0.86 | 1.13 | 0.49 | 2.39 |
| 22R503 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 9 | No | 2.11 | 1.00 | 2.00 | 13.71 | 8.80 | 45.48 | 36.30 | 0.81 | 1.27 | 0.85 | 8.71 |
| 22R504 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 25 | No | 1.80 | 1.08 | 0.41 | 11.86 | 11.43 | 39.27 | 33.05 | 0.92 | 1.19 | 0.04 | 0.38 |
| 22R505 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 4 | No | 1.75 | 1.00 | 0.00 | 13.11 | 7.72 | 44.10 | 32.53 | 0.81 | 1.36 | 0.00 | 0.00 |
| 22R506 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 12 | No | 2.92 | 1.67 | 5.20 | 19.60 | 14.31 | 50.32 | 41.75 | 0.96 | 1.21 | 2.45 | 12.94 |
| 22R506 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 25 | No | 1.92 | 1.04 | 0.21 | 15.39 | 13.74 | 48.25 | 39.22 | 0.78 | 1.24 | 0.08 | 0.97 |
| 22R507 | <i>P. edulis</i> f. <i>flavicarpa</i> × <i>P. incarnata</i> IL | 37 | No | 1.59 | 1.19 | 0.63 | 10.77 | 10.12 | 41.98 | 34.12 | 0.74 | 1.23 | 0.22 | 2.11 |

¹Feel: (feel scale of 1–5, with 1 = light, empty fruit and 5 = heavy, full fruit). ²Visual: (visual rating of pulp of 1–5, with 1 = light, empty fruit and 5 = heavy, full fruit). ³Density: $Fd = W/(H \times D)$, where W = fruit weight (g), H = fruit height (cm), and D = fruit width (cm). ⁴Shape: H/D , where H = fruit height (mm), and D = fruit width (mm). ⁵Pulp percentage [(pulp weight/total weight) × 100].

All correlations of measured traits were significant at $p < 0.05$ except for hull weight and visual assessment (Table 2). Fruit density was positively correlated with feel and total fruit weight ($r = 0.7677$ and 0.7818 , respectively). This would indicate that any of these three non-destructive measures could potentially be used equally. Total weight was highly correlated with seed number ($r = 0.8095$), pulp weight ($r = 0.7853$), and pulp percentage ($r = 0.7314$). Feel was also correlated with those same variables but lower ($r = 0.6524$, $r = 0.6592$, and $r = 0.6584$). Fruit density was similar to feel ($r = 0.6581$, $r = 0.6604$, and $r = 0.6306$). Therefore, total fruit weight, as a non-destructive measure, was the best indicator of seed number, pulp weight, and pulp percentage in this study (Table 2).

When it came to influence of male parent on traits, IL was clearly behind both MS and OK (Table 3). Vines with IL as a parent produced fewer seeds, lower total fruit weight, shorter height, smaller width, lower fruit density, lighter pulp weight and pulp percentage. Those vines with OK as a parent made more seeds, longer height, and a more elongated shape than the other two pollen parents. MS-based vines had the greatest average total fruit weight, hull weight, fruit width, and fruit density. MS and OK were not different in pulp weight and pulp percentage.

After selections were made, the individual selections kept were significantly higher/better in all traits categories except for fruit width ($p = 0.3698$) and hull weight ($p = 0.2415$) (Table 4). Eleven out of 35 vines were chosen as advanced selections.

Those chosen to advance had much higher seed numbers and pulp weights, which are highly correlated ($r = 0.9652$). Fruit that was full of pulp had the highest priority in the selection process and that was borne out in the results (Table 4).

Discussion

Making selections in a new breeding program is challenging, especially when the crop is new or underutilized. The priority was to identify the most important traits to move a vine from general population to improved selection. In the case of interspecific hybrid passionfruit (i.e., *Passiflora edulis* x *P. incarnata*), there is insufficient literature to rely upon to establish fruit quality parameters. While cold hardiness is the primary goal, other traits are also important and easier to assess without being destructive and a level of increased cold tolerance is assumed at this stage. Based on prior observation of earlier populations of interspecific seedlings and knowledge regarding self-incompatibility in both *Passiflora* species, the most important trait to select for was fully filled fruit. Poor pollination can lead to fruit that has normal size but has few or no pulp or seeds^[17]. Since the flowers were not hand-pollinated and pollination relied solely on insect pollinator activity, vines that produced well-filled fruit would be viewed as desirable in contrast to fruit that were only partially filled. The second consideration was fruit size. Average fruit size for *P. incarnata* can vary but may range between 30 and 40 g but may get to

Table 2. Correlations of fruit characteristics among all interspecific *Passiflora* hybrids selections in 2022.

| | Fruit feel | Visual | Seed # | Hull wt (g) | Pulp wt (g) | Pulp % | Density (g/cm ²) | Total wt (g) |
|-------------------------|------------|--------|--------|-------------|-------------|--------|------------------------------|--------------|
| Fruit Feel ¹ | 1.00 | 0.6586 | 0.6524 | 0.5129 | 0.6592 | 0.6584 | 0.7677 | 0.7084 |
| Visual ² | 0.6586 | 1.00 | 0.6807 | 0.0442 | 0.7215 | 0.6586 | 0.5145 | 0.3972 |
| Seed # | 0.6524 | 0.6807 | 1.00 | 0.3441 | 0.9652 | 0.9069 | 0.5681 | 0.8095 |
| Hull wt (g) | 0.5129 | 0.0442 | 0.3441 | 1.00 | 0.3073 | 0.2260 | 0.6114 | 0.8024 |
| Pulp wt (g) | 0.6592 | 0.7215 | 0.9652 | 0.3073 | 1.00 | 0.8916 | 0.6604 | 0.7853 |
| Pulp % ³ | 0.6584 | 0.6586 | 0.9069 | 0.2260 | 0.8916 | 1.00 | 0.6306 | 0.7314 |
| Density ⁴ | 0.7677 | 0.5145 | 0.6581 | 0.6114 | 0.6604 | 0.6306 | 1.00 | 0.7818 |
| Total wt (g) | 0.7084 | 0.3972 | 0.8095 | 0.8024 | 0.7853 | 0.7314 | 0.7818 | 1.00 |

¹ Feel: (feel scale of 1–5, with 1 = light, empty fruit and 5 = heavy, full fruit). ² Visual: (visual rating of pulp of 1–5, with 1 = light, empty fruit and 5 = heavy, full fruit). ³ Pulp percentage [(pulp weight/total weight) × 100]. ⁴ Density: $Fd = W/(H \times D)$, where W = fruit weight (g), H = fruit height (cm), and D = fruit width (cm).

Table 3. Fruit measurements by *Passiflora incarnata* male parent among all interspecific hybrid selections in 2022.

| Male | Total wt (g) | Hull wt (g) | Height (mm) | Width (mm) | Density (g/cm ²) ² | Shape ³ | Pulp Wt (g) | Pulp % ⁴ | Seed # |
|----------------------|--------------|-------------|-------------|------------|---|--------------------|-------------|---------------------|---------|
| IL | 14.8 c | 11.5 b | 45.9 c | 37.6 c | 0.83 c | 1.23 c | 2.00 b | 11.9 b | 5.0 c |
| MS | 22.1 a | 14.8 a | 51.6 b | 41.2 a | 1.02 a | 1.26 b | 5.24 a | 23.8 a | 10.3 b |
| OK | 19.5 b | 12.3 b | 54.1 a | 38.8 b | 0.89 b | 1.40 a | 5.90 a | 27.2 a | 14.3 a |
| P value ¹ | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

¹ Means within a column followed by the same letter are similar according to Tukey's honest significant difference (HSD) at $p \leq 0.05$. Means followed with different letters within a column are significantly different. ² Density: $Fd = W/(H \times D)$, where W = fruit weight (g), H = fruit height (cm), and D = fruit width (cm). ³ Shape: H/D, where H = fruit height (mm), and D = fruit width (mm). ⁴ Pulp percentage: [(pulp weight/total weight) × 100].

Table 4. Resulting traits of interspecific *Passiflora* vine selections that were kept and those discarded during the 2022 season.

| | Seed # | Total wt (g) | Height (mm) | Width (mm) | Density (g/cm ²) ² | Shape ³ | Pulp wt (g) | Hull wt (g) |
|----------------------|---------|--------------|-------------|------------|---|--------------------|-------------|-------------|
| Kept | 18.2 | 21.7 | 54.3 | 39.0 | 0.97 | 1.40 | 7.5 | 12.6 |
| Discarded | 7.0 | 16.9 | 50.4 | 38.7 | 0.84 | 1.31 | 3.0 | 12.2 |
| P value ¹ | <0.0001 | <0.0001 | <0.0001 | 0.3698 | <0.0001 | <0.0001 | <0.0001 | 0.2415 |

¹ Means within a column are different according to Tukey's honest significant difference (HSD) at $p \leq 0.05$. ² Density: $Fd = W/(H \times D)$, where W = fruit weight (g), H = fruit height (cm), and D = fruit width (cm). ³ Shape: H/D, where H = fruit height (mm), and D = fruit width (mm).



Fig. 2 Fruit from *P. edulis* (purple and yellow) (left), *P. incarnata* (middle) and hybrid fruit (right). Hybrid fruit remained green similar to the *P. incarnata* parent.

nearly 60 g^[10]. *Passiflora edulis* also has a wide range depending on type. Purple fruit is usually far smaller than yellow or red fruit (Fig. 2), ranging from ~80g for purple to ~150 or more for yellow and red^[18–19]. Since these species were crossed, one would expect to see intermediate traits, including fruit weight; however, this was not the case. Fruit weights were smaller than either parent (Table 1), although fruit from some selections approached that of *P. incarnata*. Arjona et al.^[20] reported decreased fruit weight in container-grown *P. incarnata* vines in a greenhouse. Knight^[11] found a wide range of fruit weights among *Passiflora* hybrids, some results similar to what was observed in the present study. However, in the study by Knight^[11] the vines were tetraploid and thus were able to achieve larger size and heavier fruit weights. Conditions reported in other studies likely differed substantially from this one, yet the small fruit weight is a concern. It is important to have fruit that is not too small, such that consumers find it potentially a poor value. Because fruit fill and fruit size were the most important overriding factors in advancing selections in this study, other traits such as sugar and acid levels were not considered but will be in the next round of selection.

Fruit fill cannot be determined solely based on visual assessment of the whole fruit, nor by total fruit weight. Percent pulp can be measured via destructive measurement, but this is time consuming. A physical assessment of fruit fill can be done without measuring the amount of pulp, but it is subject to unmeasurable variables such as hull weight and thickness that make feel-based ratings a potentially inaccurate metric. Therefore, a non-destructive method to aid in the selection making process would be extremely useful. Fruit density proved to be similar to other methods (Table 2) for estimating fruit fill and could have potential as more data are collected to determine its appropriateness.

Fruit shape was a less important consideration in the selection process as well. Most of the fruit was oblong, although not extremely so. Most *P. edulis* fruit are round or near-round and therefore that shape is the accepted standard for passionfruit^[21]. With backcrossing, future selections may come closer to the round shape that is expected of passionfruit, but as the American consumer by-and-large has little knowledge of passionfruit it may be possible to introduce other fruit shapes into the marketplace.

High seed number is desirable because an aril must have a seed to form (there are currently no seedless passionfruit although the possibility is being explored^[22]), but seeds of *P.*

incarnata are larger and harder than *P. edulis*. Hybrids produced intermediate sized seeds, but they were still considerably more noticeable than *P. edulis* seeds. *Passiflora edulis* seeds can be readily consumed, as they are small enough to not be noticed. Separating the seeds from the pulp is labor intensive as well. The future of passionfruit production in the US is in fresh fruit consumption and not the processing market due to the relatively inexpensive volume of juice and pulp that is imported from South America^[22].

Breeding with wild or native plant material can be a long-term endeavor. Numerous challenges exist within *Passiflora* that make progress difficult, especially in interspecific breeding efforts with *P. incarnata*. These obstacles include large seed size, poor fruit fill (poor pollination, self-incompatibility), low fruit quality, smaller fruit size, and short postharvest shelf life.

One big advantage for using *P. incarnata* is the improvement in cold hardiness^[11,13,19,23]. While there are problems with incompatibility and hybrid sterility^[10,23] when using this species, successful crosses can be made. Since *P. incarnata* is an herbaceous vine, it will die to the ground starting in fall. Yet, early in the following spring it will emerge from the roots and within weeks it will produce flower buds. Conversely, the *P. edulis* vine is woody and can be severely damaged by cold temperatures. In south Mississippi, vines of *P. edulis* f. *flavicarpa* were completely killed by temperatures of 22 °F (–5.6 °C) even when grown under a high tunnel structure. Interspecific hybrid vines show intermediate growth habit, partially dying back in fall and winter. This could potentially leave them susceptible to damage at low temperatures aboveground. Observations of interspecific hybrids vines in south Mississippi have shown them to vary in their ability to handle cold temperatures, with some dying and some returning strongly from the roots. Appropriate selection for cold hardiness will be needed to progress breeding efforts.

Conclusions

There is reason to be cautiously optimistic about interspecific hybrid *Passiflora* involving *P. incarnata*. However, as seen in previous studies, the challenges are significant toward the production of a commercially viable fruit. In the case of this study, the fruit obtained was smaller than desired with lesser fruit quality than that of *P. edulis*. Therefore, creating more generations with backcrossing to *P. edulis* is the next logical step in the process with the expectation of incremental losses

of cold hardiness. Additional proxy experiments of cold hardiness, such as differential thermal analysis and electrolyte leakage^[24] will be performed as it becomes necessary to determine more precise estimates of tolerances to cold temperatures.

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Conflict of interest

The author declares that there is no conflict of interest.

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