ELSEVIER

Industrial Crops and Products 12 (2000) 151–157

INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL

www.elsevier.com/locate/indcrop

Simmondsin and wax ester levels in 100 high-yielding jojoba clones

Hal C. Purcell [a,*], Thomas P. Abbott [b], Ronald A. Holser [b], Bliss S. Phillips [b]

[a] *Purcell Jojoba International, LLC, PO Box 659, 142 Front Street, Avila Beach, CA 93424, USA*

[b] *New Crops Research, National Center for Agricultural Utilization Research, Agricultural Research Service, USDA, 1815 North University Street, Peoria, IL 61604, USA*

Accepted 12 May 2000

Abstract

This 3-year study examines differences in simmondsin and wax ester production by 100 previously identified high-yielding jojoba clones. Over the past 3 years the USDA, ARS, National Center for Agricultural Utilization Research (NCAUR) analyzed seed samples from Purcell Jojoba International's (PJI) large 17-year-old Variety Trial program. These 100 clones were the top producers out of 1523 clones that had been mass-selected from more than 1.5 million, open-pollinated, female plants. Broad selection criteria were applied to retain a healthy degree of the natural plants' heterogeneity and hybrid vigor. In this study, one female clone produced 82% higher simmondsin levels than the mean of the 100 clones analyzed. Another clone produced 16% higher liquid wax ester levels than industry average. A few clones had high levels of both simmondsins and esters. In addition, the differences in fatty acid and fatty alcohol among the clones were also studied. These analyses help provide a strong foundation for major growth of the jojoba industry. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Jojoba; *Simmondsia chinensis* (Link) Schneider; Wax esters; Simmondsin; Appetite suppression; New crop

1. Introduction

Successful domestication of a wild plant and development of markets for its new products requires close cooperative research efforts between public and private organizations (Princen and Rothfus, 1984). Systematic germplasm collection, clonal evaluation, variety trials, and cultivar selection are the keys to successful jojoba production (Purcell and Purcell, 1988). These two statements describe the processes that made this work possible.

Jojoba seeds are the economic product of this wild, perennial, desert plant. They contain an average of 50% liquid material that is a unique array of 98% pure wax esters. All other known seed oils are essentially triacylglycerols with only very small amounts of wax esters. A wide diversity of plants, most of which do not have good yields, grow from jojoba's highly heterogeneous wild seeds. Nature has perfected jojoba over thou-

* Corresponding author. Tel.: +1-805-5957275; fax: +1-805-5959138.

PII: S0926-6690(00)00066-2

sands of years. Every year hundreds of billions of new recombinations of jojoba DNA are put to a 'life or death' survival test in the hot dry desert. Jojoba plants are dioecious. The pollen of the male trees is scattered for miles by the wind. Only female trees reproduce seeds (Gentry, 1958). This outbreeding has resulted in highly heterogeneous seeds that provide us with a wide range of hybrid vigor and fertility. Ironically, this same extreme genetic variation that was a major cause of failure in the seed-planted fields of the early jojoba pioneers, is also a major key to high yields for the future. Lack of understanding of floral biology also contributed to their failure.

The first jojoba selection and propagation from wild plants in the North American Sonora Desert, the only native habitat of jojoba, was done in the 1950s to 1970s by Drs Yermanos, Hogan, Palzkill and others. It was difficult to compare native wild plants due to their widespread locations and unknown variables such as plant age, water availability, soil type, microclimate and proximity of male pollinators. Despite these obstacles, seeds and cuttings collected from ~ 2000 wild jojoba plants were planted in university research plots. Average yields from these first selections were ~ 600 g per plant by the 7th year (Palzkill and Hogan, 1982; Yermanos, 1982). During the 1980s, Purcell Jojoba International, LLC (PJI) inspected millions of Sonora Desert farm-cultivated, same-aged, seed-propagated jojoba plants growing in rows with uniform spacing, soil and irrigation conditions. These source plants had not been replicated, but did provide a good general idea about long-term performance and tolerance of extreme environmental events, such as frost, floods and insects. PJI collected a broad spectrum of vigorous, high-yielding genotypes for its Sonora Desert variety trials before these commercial fields were abandoned. PJI planted replicated Variety Trials with 1523 jojoba clones that had been selected for high yields and manageability from more than 1.5 million cultivated seedings. One early source of germplasm was PJI's 19-year-old field where 300 plants have been hand-harvested (Fig. 1) and tested at the University of California for ester content. The over-all 12-year mean yield for these plants was 1369 ± 36.9 g per plant with an average wax ester level of $51.5\% \pm 0.13$. At the same time that PJI was selecting for high-yielding plants, other researchers were isolating and purifying simmondsin from jojoba meal (Abbott et al., 1991, 1994; Erhan et al., 1997). In 1992, Cokelaere published a report that jojoba simmondsin might reduce food intake (Cokelaere et al., 1992). In 1994, PJI entered into a CRADA with the USDA NCAUR to identify which of PJI's 100 highest seed-yielding clones also have qualitative and quantitative differences in esters and simmondsins. Genotypes have now been identified with the potential to double yields of seeds with 16% higher levels of esters and 82% higher levels of simmondsins.

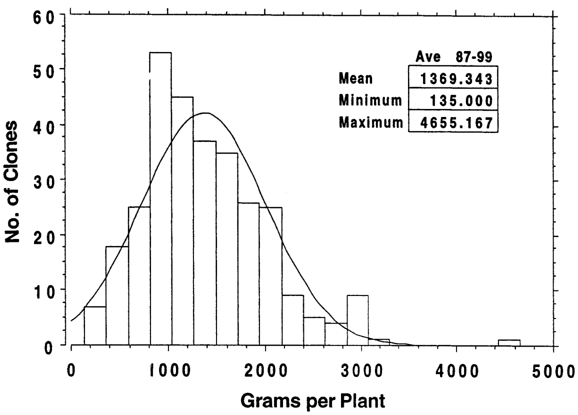


Fig. 1. Distribution of 12-year means of 300 hand-harvested plants.

2. Materials and methods

PJI chose 1523 genotypes (clones) for asexual stem cutting propagation. Twenty genetic duplicates of each genotype were propagated for a total of 30 460 trees. In 1986 these cuttings were transplanted into the variety trials where each of four replications contains five of each of the 1523 genotypes for a total of 7615 trees per replication. In 1995 100 top seed yielding clones were selected for research with the USDA.

Plant canopy volume, seed size, bloom time, seed drop and other traits were recorded. However, because the cost of an annual hand harvest

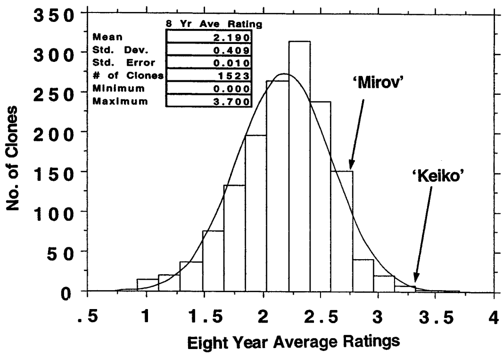


Fig. 2. Eight-year averaged ratings (1, low to 5, high) of yields of 1523 clones.

of 30 460 plants would be prohibitive, rather than greatly reduce the number of clones, a more cost-effective procedure to estimate yield levels was devised. A trained observer rated each plant on a 1 (low yield) to 5 (high yield) scale for seed yields each year since 1992. These estimates of relative yields were used to compare and rank the genotypes in this study.

To evaluate the liquid wax ester levels and composition, the jojoba seeds were extracted using a Butt extraction method with petroleum ether as solvent for 24 h. Extracted wax esters were analyzed by short column GC to determine the wax components of each extract and then transesterified to determine the ratio of alcohols and acids composing the wax extract, but these results will be reported elsewhere.

For simmondsin analyses, one tenth gram samples of ground defatted jojoba meal were weighed into a glass vial and mixed with 10 ml of deionized water containing 1 mg/ml benzyl alcohol as internal standard. The sample was sonicated for 90 s with a model VC100 VibraCell sonicator (Sonics and Materials, Danbury, CT) using an amplitude setting of 50 and a 6-s pulse. The solids were allowed to settle and the liquid portion was withdrawn by syringe and filtered through a disposable 0.45 μm filter unit (Millex-SR, Millipore) to clarify the liquid extract in preparation for analysis.

Samples were analyzed on a 250 mm × 4.6 mm Luna 5 μm C18 column (Phenomenex, Torrance, CA) using a Spectra Systems 2000 (Thermo Separations, San Jose, CA) high performance liquid chromatography. The solvent gradient of 100% water to 100% methanol in 20 min at a flow rate of 0.75 ml/min gave a total analysis and equilibration time of 40 min per sample. An injection volume of 5 μl was used. All analyses were run in duplicate with 1 mg/ml benzyl alcohol as an internal standard. Detection was achieved by UV absorption at 220 nm in a 1-cm path length flow cell. Solvents were analytical grade and degassed with helium prior to use.

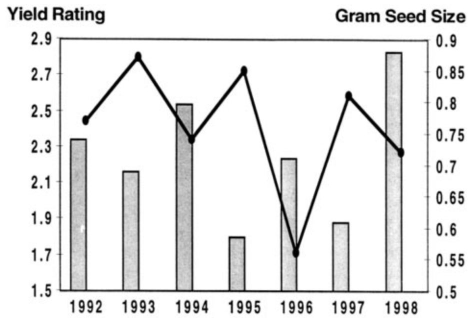
3. Results

3.1. Yields and alternate-bearing

The overall 8-year mean yield rating from replicated clones was 2.16 ± 0.01 on a 1–5 scale (Fig. 2). The mean yield rating for 'on years' was 2.46 ± 0.022 and for alternate 'off years' was 1.88 ± 0.018. Generally speaking, in the years of high production, the average clone yielded ~ 1800 g and high-yielding clones produced over 7000 g. In alternate, low-producing years, individual clone yields ranged from 10 to 50% as much.

Growers of commercial fields have often erroneously concluded that jojoba is not alternate bearing. In their seed-planted or mixed-cutting planted fields some plants are in the heavy producing over-bearing cycle and some in the low-producing, underbearing cycle in a given year so that the yields average out. The issue is further obscured by variance in the alternate bearing yield pattern among and within clones. Some plants within a clone were found to be on opposite cycles in the same year. Some clones produced high yields for 2 or 3 years, then had low yields the next year. Others had low yields for a few years then had a high yield the next year. The plants within a clone generally become more synchronized as they mature. When individual plants were followed over 10–15 years, the alternate-bearing phenomenon was obvious and prevalent in the great majority of our clones.

High-yielding plants with larger seeds appeal to jojoba plant selectors because larger seeds are easier to harvest and handle. Certain female clones produce much larger seeds, but the seed size is also influenced by alternate bearing and environmental factors such as temperatures and water stress. Alternate bearing affects jojoba seed size (Fig. 3) in the same way it does other plant species. Over-bearing jojoba plants produce smaller seeds and under-bearing plants produce larger seeds.



	1992	1993	1994	1995	1996	1997	1998
Ave Yield Rate	2.34	2.16	2.54	1.8	2.24	1.88	2.83
Std. Error	0.03	0.03	0.03	0.02	0.03	0.02	0.02
Ave Seed Wt	0.77	0.87	0.74	0.85	0.56	0.81	0.72
Std Error	0.02	0.02	0.03	0.02	0.02	0.02	0.02

Fig. 3. Alternate-bearing affects the overall average annual seed size in the variety trial. Note the sunburn's at least additive effect of lower than expected yields and seed size in 1996.

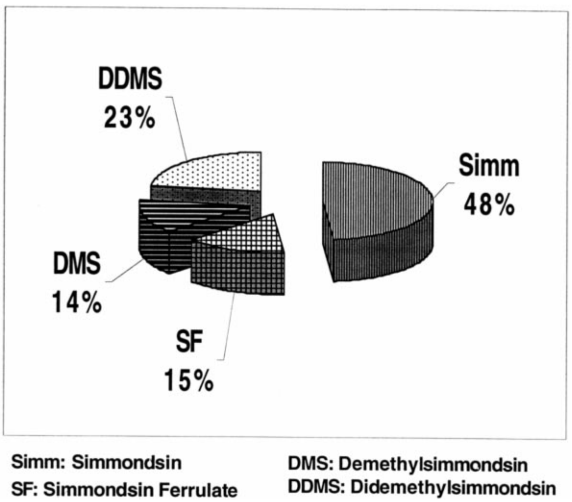


Fig. 4. Simmondsin and three analogs were found in these average percentages over the 3-year analysis of PJI jojoba meal.

A dramatic environmental event occurred during May of 1996 when a new Arizona, all-time, high temperature record of 53.3°C. was set. The heat and actinic radiation from direct sunlight burned exposed surfaces of leaves, stems and fruits black. Development of seeds, particularly in late blooming plants was arrested, resulting in many smaller immature seeds and lower yields.

3.2. Selection for wax esters

A 50% liquid wax ester level in seeds from wild and cultivated jojoba is accepted as the jojoba industry base average from which seed prices have been adjusted for higher or lower ester levels (Purcell, 1984). By eliminating lower ester level clones over the past 12 years the PJI average ester level has increased to over 52% in commercial fields.

PJI's latest 3-year-averages of ester levels in 100 clones measured by the USDA had an overall mean of $54.1 \pm 0.14\%$. The top six clones in this USDA study had a mean of 57.5% esters. One clone's mean over 3 years was 58% and the highest single measurement was 59.9%.

Regression analysis attempting to predict the 1998 ester levels from an average of the 1996 and 1997 same-plant levels resulted in a 0.154 adjusted r^2 value. A few of the external factors that could affect ester levels are variance in the growing season, alternate bearing, seed size and pollen source.

Regression analysis of 460 UC Riverside measurements and 292 USDA measurements indicated little or no relationship between hereditary seed weight and ester levels. The UC Riverside 3-year study had an adjusted r^2 of 0.053 and the USDA 3-year study's r^2 was 0.095. Most of this USDA coefficient of determination came from a r^2 of 0.23 in 1996, the year of premature arrest in seed and ester development.

3.3. Selection for simmondsins

The USDA also measured simmondsin and simmondsin analog levels in PJI's top 100 clones (Fig. 4). The four types of simmondsins measured were simmondsin, simmondsin ferrulate,

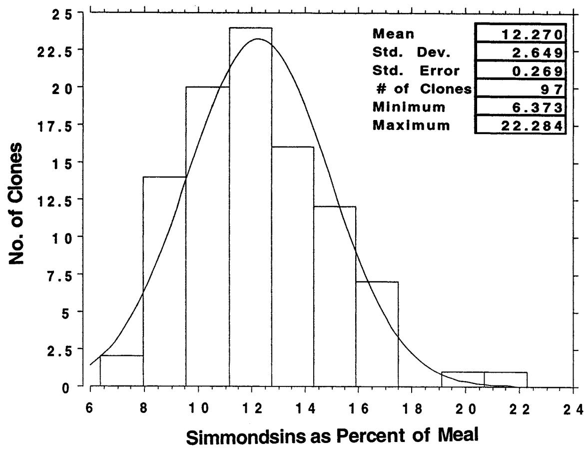


Fig. 5. Simmondsins content of 97 clones (means for 1996, 1997 and 1998 crops).

desmethylsimmondsin (DMS) and dedesmethylsimmondsin (DDMS). There are some clonal differences in the levels of the various types of simmondsins, but this report is on total simmondsins.

It is important to distinguish between percent simmondsins in meal and in the seed. The former is measured and the latter is a calculated adjustment for the ester levels in the seed. Plant selectors are more interested in the percent simmondsins in the seed, since it denotes absolute simmondsin production. Food scientists and nutritionists are more interested in simmondsin levels in the meal as they prescribe dosages for their diet formulas. Reported here are the simmondsin levels in the meal.

The mean of three laboratory measurements of total simmondsins were made for each clone for each year, then the three mean values were averaged (Fig. 5). The 3-year average of individual clone means ranged from 6.4 to 22.3% with an overall mean of $12.2 \pm 2.62\%$. Clone number 4018 had seed yields of about double the industry average and average total simmondsin levels of 22.28%, which is 82% above our average for 100 clones. Based on its higher yields and simmondsin levels, this clone has the potential to produce more than three times as much simmondsin as the industry average.

Utilizing regression analysis, the mean simmondsin levels of 1996 and 1997 were used to predict the 1998 simmondsin level. The regression analysis r value was 45.2 and r^2 was 20.5.

4. Discussion and conclusions

The female genetic contribution to yields is very important, but many other factors such as alternate bearing and environmental interactions including growing seasons and cultural practices also affect annual yields and seed sizes. Randomized, replicated variety trials treated with current good cultural practices were utilized to help separate genetic traits from environmental effects.

Genetic selection is the key to long term success in jojoba production. Selection from variety trials with a wide range of plant genotypes provides a basis to ‘design’ ideal cultivars as needed for new cultural practices or emergence of new products like simmondsin. An example of the selection process follows.

These 15 top choices were selected from PJI Variety Trial clones that came from millions of cultivated plants (Table 1). All 15 ranked among the 2–3% highest yielding clones in the PJI Variety Trial over the past 8 years. One has a simmondsin level of 22.24% that is 82% above the overall average. One has an ester level of 58% that is 16% above the industry average.

In the mid 1980s PJI selected and propagated the ‘Keiko’ clone from its initial plantings and the ‘Mirov’ clone from a 1968 University of California planting near Riverside, CA. These two clones were used as check plants for PJI research plots. The ‘Mirov’ was also transferred to growers in the US and other countries. By the late 1980s the University of California found each clone also had high ester levels.

The upright growth, basal clearance and large seeds made ‘Keiko’ the first choice for PJI’s ‘from-the-ground’ harvesting system first introduced in 1987 (Carnegie and Purcell, 1988). Between 1989 and 1994 PJI propagated and transplanted over 200 000 ‘Keiko’ plants in three commercial fields each with a different spacing configuration. The current USDA–PJI research

Table 1
Top 15 PJI clone selections based on manageability, yields, ester levels and simmondsin levels

ID	3-year ave. seed wt.	3-year ave. esters (%)	3-year ave. simmondsins (%)	8-year ave. yield rating
5004	0.79	58.0	9.0	3.38
2005	0.88	57.9	11.8	3.30
2017	0.79	57.7	12.5	3.05
3735	0.80	57.0	8.1	3.00
8011	1.08	57.0	15.2	3.52
7011 'Keiko'	0.86	56.8	14.1	3.26
6005	0.67	55.7	15.9	3.06
7013 'Mirov'	0.64	55.5	15.0	2.70
4002	0.82	54.7	17.2	3.49
6007	0.75	54.3	16.5	2.64
3006	0.86	54.0	16.6	3.07
8010	0.87	53.5	11.3	3.61
1008	1.00	53.3	19.5	3.00
4010	0.76	53.0	16.3	2.68
4018	0.55	51.7	22.3	2.79
100 Clone ave.	0.77	54.3	12.2	3.03

confirmed high ester levels for 'Keiko' and 'Mirov' (Fig. 2 and Table 1). 'Mirov' has ranked 87th in yield ratings over the past 8 years.

'Keiko' currently ranks 6th out of 1523 clones in yields and it ranks 6th out of the top 100 USDA study clones with 56.8% in ester levels. This amounts to a 13.6% higher ester level than the industry average of 50%. The 'Keiko' cultivar ranked 23rd out of 100 with 14.14% simmondsins. The value of simmondsin will depend on the results of additional biological testing.

Over the past 18 years PJI carried out extensive mass selection from millions of female jojoba plants to select 1523 clones for randomized replicated variety trials. Broad selection criteria were applied to retain a healthy degree of the wild plants' heterogeneous vigor. The University of California screened these plants for ester levels in the late 1980s. Yields have now been rated for 8 years. In this current study, seeds from the 100 highest yielding plants were further analyzed by the NCAUR for high ester levels, simmondsin levels and ester profiles.

Clones have been identified with 100% higher yields, 16% higher ester levels and 80% higher simmondsin levels than the respective means. These analyses will help provide a strong foundation for major growth of the jojoba industry.

Acknowledgements

The USDA's New Crops Research Unit of the Agricultural Research Service's National Center for Agricultural Utilization Research, the University of California and Purcell Jojoba International all made significant contributions to this project through cooperative research agreements.

References

Abbott, T.P., Nakamura, L.K., Buchholz, G., Wolf, W.J., Palmer, D.M., Gasdorf, H.J., Nelsen, T.C., Kleiman, R., 1991. Processes for making animal feed and protein isolates from jojoba meal. J. Agric. Food Chem. 39, 1488–1493.

Abbott, T.P., Nabetani, H., Kleiman, R., 1994. Processing jojoba meal for value added products by membrane separation. In: Princen, L.H., Rossi, C. (Eds.), Proceedings of the IX International Conference on Jojoba and Its Uses. American Oil Chemist Society, Champaign, IL, pp. 126–130.

Carnegie, E.J., Purcell, M., 1988. Evaluation of jojoba harvesting systems. In: Baldwin, A.R. (Ed.), VII International Conference on Jojoba and Its Uses. American Oil Chemist Society, Champaign, IL, pp. 123–132.

Cokelaere, M.M., Dangreau, H.D., Arnouots, S., Kuhn, E.R., Decuypere, E.M.P., 1992. Influence of pure simmondsin on food intake in rats. J. Agric. Food Chem. 40, 1839–1842.

Erhan, S.M., Abbott, T.P., Nabetani, H., Purcell, H.C., 1997. Simmondsin concentrate from defatted jojoba meal. Ind. Crops Prod. 6, 147–154.

Gentry, H.S., 1958. The natural history of jojoba. Econ. Botany 12, 261–295.

Palzkill, D.A., Hogan, L., 1982. Importance of selection and evaluation of vegetatively propagated jojoba before commercial release. In: Elias-Cenik, A. (Ed.), Jojoba and Its Uses Through 1982, Proceedings of the Fifth International Conference. University of Arizona, Tucson, AZ, pp. 177–179.

Princen, L.H., Rothfus, J.A., 1984. Development of new crops for industrial raw materials. J. Am. Oil Chem. Soc. 61, 281–289.

Purcell, H.C., 1984. Jojoba Growers Association's jojoba seed and oil standards committee progress. In: Wisniak, J., Zabicky, J. (Eds.), Proceedings of the Sixth International Conference on Jojoba and Its Uses. Ben Gurion University of the Negev, Beer-Sheva, Israel, pp. 299–303.

Purcell, H.C., Purcell, H.C. II, 1988. Jojoba crop improvement through genetics. In: Baldwin, A.R. (Ed.), VII International Conference on Jojoba and Its Uses. American Oil Chemist Society, Champaign, IL, pp. 69–85.

Yermanos, D.M., 1982. Performance of jojoba under cultivation between 1973 and 1982, information developed at the University of California, Riverside. In: Elias-Cenik, A. (Ed.), Jojoba and Its Uses Through 1982, Proceedings of the Fifth International Conference. University of Arizona, Tucson, AZ, pp. 200–201.