



RESEARCH FOCUS

Small but Mighty: 'Pixie' Grapevine Speeds Up the Pace of Grape Genetics Research and Breeding

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The dwarf 'Pixie' vine continually produces flower clusters instead of tendrils, providing breeders and geneticists with a new tool for speeding up breeding and reducing the costs associated with genetic studies.

Photo by Joe Ogrodnik

Grape research can be experimentally inconvenient. The vines take up a lot of space, and half the year they are dormant and unavailable for study. Grape geneticists and breeders who want to evaluate traits involving fruit—whether it's fruit chemistry, bunch rots, or physiology—are constrained by the seasonal availability of fruit, and creating new varieties or populations for study or evaluation is slowed by the time it takes for a seedling to bear fruit. In New York's climate, it can take three or more growing seasons for a vine to fruit. We report on unique new varieties developed in Geneva that are well-suited for research and breeding because they flower and bear fruit year round and are compact enough to thrive in a greenhouse.

KEY CONCEPTS

- Grapevines have a long generation time. From seedling to flowering can take three years.
- Grapevines for genetic research occupy a lot of space and are expensive to maintain.
- Both the expense and generation time limit breeding and genetics studies.
- The new dwarf 'Pixie' vine grows in a small pot and continually produces flower clusters.
- Continuous flowering and seed production can shorten the generation time from three years to less than 200 days.
- This allows breeders and geneticists to perform genetics studies using 'Pixie' in months rather than the several years require for studies using standard-sized grapevines.
- 'Pixie' will speed up the process of matching vine traits to specific genes and patterns of inheritance.
- 'Pixie' was derived from Pinot Meunier, itself a naturally occurring mutant of Pinot noir with two genetically distinct cell layers.

Progress in grape breeding progress and genetics research can be hindered by the size and generation time of our favorite plant—the grapevine! In addition to being a lot larger than many other familiar crop plants—amplifying the space and resources required for research—grapevines take years to reach sexual maturity, delaying the production of the fruit that is essential for evaluating a new selection. It can take several years from the time that a nursery grown vine is planted until it begins to yield, and growing grapevines from seed can add another year or more in many climates. Grape breeding and genetics research is still constrained by size and generation time relative to many other crops.

The ‘Pixie’ vine. The ‘Pixie’ grapevine is a new, dwarf variety developed by the USDA Agricultural Research Service (USDA ARS) in Geneva which preferentially produces inflorescences (flower clusters) instead of tendrils, even as seedling (See figure on p. 1). In addition, the internode length (distance between leaf nodes) is quite short, resulting in a vine compact enough to be grown to flowering and fruiting in a four inch square pot in a greenhouse. It was derived from Pinot Meunier using tissue culture by the USDA ARS in collaboration with the Ralph M. Parsons Foundation Plant Transformation Facility at the University of California, Davis (see sidebar).

A natural, gibberellin mutant. ‘Pixie’ gets its small stature and juvenile, continuous flowering courtesy of one copy of a naturally occurring, mutated gene from Pinot Meunier which reduces its sensitivity to the plant hormone gibberellic acid. Gibberellic acid has diverse effects in grapevines, including determining whether the structure across the shoot from a leaf will be an inflorescence (cluster or bunch) or a tendril. Tendrils are a derived form of inflorescences which have lost their flowers and become highly adapted for clinging. The influence of gibberellic acid on the number of clusters per shoot is akin to easing up on a gas pedal; adding gibberellic acid eases up on the pedal and we get fewer clusters and more tendrils, while block-



Photo by Peter Cousins

Fruit clusters on a single shoot of a ‘Pixie’ vine. Note the range in stages of development of the different clusters.

ing gibberellic acid or taking it out of the system increases the number of clusters and decreases the tendrils. ‘Pixie’ vines are insensitive to gibberellic acid and therefore produce only inflorescences and no tendrils at all.

Putting ‘Pixie’ to work. ‘Pixie’ was released in 2006 as a public variety without intellectual property protection. Since then, cuttings, seeds, and plants have been distributed to researchers across the United States and internationally, including Chile, Hungary, Germany, and Switzerland, for use in research and breeding. ‘Pixie’ vines can simultaneously bear shoots with flower buds, flowers in bloom, immature fruit, fruit at veraison, and ripe fruit. Therefore, with a sufficient number of vines, fruit and flowers at all stages of development are available throughout the year, providing opportunities for researchers in many disciplines to investigate growth, development, reproduction, and fruit composition year round. ‘Pixie’ has been used by food science researchers to investigate the chemistry of developing grape berries and by plant pathologists to examine resistance to powdery mildew regardless of season.

Origin of the ‘Pixie’ vine

‘Pixie’ vines were developed from the wine grape variety Pinot Meunier using tissue culture. Pinot Meunier is in many ways a typical grapevine—it is a sport of Pinot noir that has abundant white hairs on its leaves. However, Pinot Meunier is actually a chimera in which cells can be genetically different in the L1 layer (outside) and L2 layer (inside), which fits inside the L1 layer like a hand in a glove. In Pinot Meunier, the difference between the L1 and the L2 layers is that the L1 outside layer is hairy, and the L2 layer is essentially identical to Pinot noir.

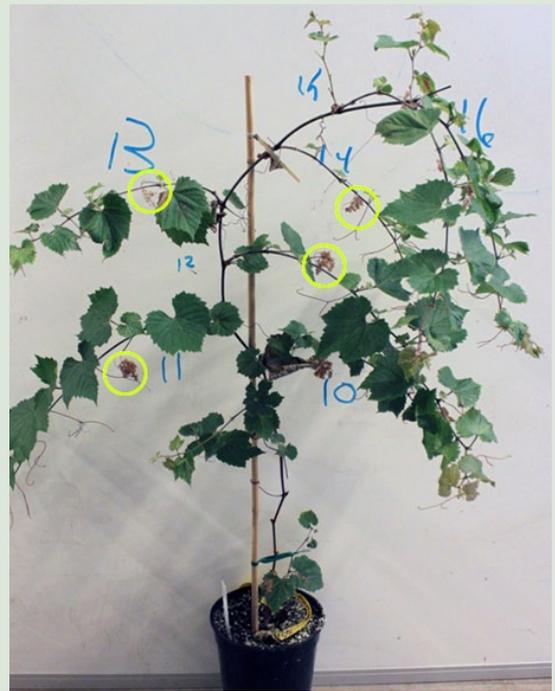
Australian researchers Skene and Barlass (1983) first reported a method for separating the L1 and L2 layers of Pinot Meunier and grew some dwarf vines, but their vines did not show the high degree of conversion of tendrils to inflorescences that are so characteristic of ‘Pixie’. Later, another team of researchers in Australia, led by Boss and Thomas (2002), used a different approach to successfully separate the L1 and L2 layers of Pinot Meunier, and their “microvines” produced from the L1 layer were dwarf vines that preferentially produced inflorescences in place of tendrils. Because moving grapevines internationally is heavily regulated due to quarantine laws aimed at reducing the distribution of harmful grape pests and pathogens, we used the same starting material as Boss and Thomas and derived a dwarf vine from Pinot Meunier in collaboration with David Tricoli of the Ralph M. Parsons Foundation Plant Transformation Facility at the University of California, Davis. ‘Pixie’ was released in 2006.

'Scout'-ing out other possibilities

Dwarf vines like 'Pixie' are just one of several approaches to accelerating grape breeding on a smaller research footprint. We are working with some wild grapes which, although not dwarf like the 'Pixie' vine, bear only inflorescences and no tendrils. Even in outdoors Geneva, the vines flower in the spring and continue all season until they are forced to stop by winter's arrival. In 2010, the USDA ARS released 'Scout', an experimental grapevine variety derived from this wild variation. 'Scout' flowers early, at approximately the tenth node — about 100 days after planting — and the vines in our greenhouse have been blooming almost without interruption since the first observations were made in 2009. In the greenhouse 'Scout' blooms continuously from prompt lateral branches, although it does make true tendrils. Cutting-grown 'Scout' vines will flower and set fruit in small pots in a greenhouse and with judicious pruning can be induced to be in bloom at any time of the year.

A seedling of 'Scout' illustrating the many inflorescences produced on the small, young plant. Flowers are being produced as low as the branch at the 10th node.

Photo by Peter Cousins



Developing new populations for breeding and genetics—faster. 'Pixie' and related dwarf vines are a useful way to reduce the time and space required for grapevine breeding and genetics research. 'Pixie' vines have one copy of a naturally occurring, mutated gene from Pinot Meunier that reduces the sensitivity of the plant to gibberellic acid. Since

'Pixie' vines have one normal copy and one mutated copy of the gene, they will pass the mutation on to half of their offspring in crosses with ordinary vines. For example, a cross of Chardonnay (ordinary size) with 'Pixie' (dwarf) would be expected to give one half dwarf vines and one half ordinary size vines. We can move ahead through breeding generations rapidly—one generation per year—while not losing the genetics that are capable of producing ordinary-sized vines.

Typically, when a grapevine seedling first begins to grow, it does not produce tendrils or inflorescences until its third growing season, but this varies with the growing conditions and the genetic background of the seedling. Dwarf vine seedlings bred from 'Pixie', however, preferentially produce inflorescences instead of tendrils. Instead of beginning to produce tendrils opposite the seventh leaf, these dwarf seedlings produce inflorescences at this position and preferentially produce inflorescences at all tendril positions thereafter. The consequence is that dwarf seedlings can start to flower within 95 days from germination in a pot in a greenhouse, while ordinary grape seedlings might require several years and a lot of vineyard space before they produce flowers. The fruit takes another 100 or so days to mature the seeds. Although the 'Pixie' vine is not as fast as a tomato or as small as a radish, the reduction in the size and acceleration in generation time are useful advances for grape breeding and genetics research.



Wild type ordinary grapevine seedling (left), heterozygous dwarf (middle), and homozygous double dwarf vines at about 90 days old; middle vine already has flower buds.

Photo by Peter Cousins

Studying pest resistance. In Geneva, the USDA Agricultural Research Service has continued to develop new dwarf grape populations for research, evaluating the value of particular selections as parents or for conducting experiments. The 'Pixie' and dwarf grapevine project grew out of the grape rootstock improvement program: we wanted to conduct lots of experiments on the inheritance of pest resistance genes, but the generation time and space requirements posed a challenge for asking those questions. 'Pixie' and dwarf vines are a new tool for us in describing the genetic control of pest resistance and enabling faster breeding of improved pest resistant rootstocks.

Tackling the genetics of fruit traits. Now that ‘Pixie’ and dwarf vines are available for breeding and genetics research, we can more easily study fruit traits that previously were lengthy and expensive to investigate. An entire segregating population of many vines can be brought to fruit in a few months on a greenhouse bench covering a few square feet, while the same population of ordinary vines in the vineyard might take years and acres to investigate. ‘Pixie’ and dwarf vines are not a substitute for all vineyard research, but they allow researchers to answer questions about fruit-related traits more rapidly and cost effectively, speeding the delivery of new varieties to grape growers and wine makers.

For example, we now have a dwarf vine that carries root-knot nematode resistance from the rootstock ‘Freedom’, and we can use this dwarf vine as founders for rapid breeding rootstock populations, combining sources of resistance into elite selections. We have pistillate (female) flowered dwarf lines to facilitate cross breeding (no emasculation needed) and white fruited dwarf lines for genetics of fruit color studies.

Carbon partitioning. Dwarf plants can highlight important aspects of flowering, fruitset, and ripening. On an ordinary grapevine, the ratio of leaves to fruit increases as the season progresses. In the vineyard the number of clusters is typically set during bunch initiation the previous year. Each shoot has a given number of clusters, and the number of leaves increases throughout the season as the primary shoot and lateral shoots grow and produce new leaves. In contrast, ‘Pixie’ vines have an essentially fixed ratio of fruit to leaves, usually two clusters for every three leaves (reflecting the discontinuous pattern of tendrils and clusters relative to nodes found on most varieties, *V. labrusca* hybrids excepted).

We observe that individual shoots on dwarf vines tend to grow and flower strongly to a point, then when the older clusters start to ripen the growth of the shoot and production of new inflorescences slows substantially or can even halt altogether. This indicates the possibility of using dwarf vines for studying the genetic and environmental influences on carbon partitioning in vines. ‘Pixie’ vines start off with a very high level of fertility and could be thinned down to any arbitrary number of clusters per shoot. In addition to the flowering differences, ‘Pixie’ and dwarf vines are distinguished from ordinary vines in the morphology and distribution of their roots, and this difference might be applicable to the development of pest resistant rootstocks with naturally restricted root zones (and potentially the induction of low vigor).

Next steps. Breeding rapidly cycling, dwarf, and small vines—like ‘Pixie’ and ‘Scout’ (see box)—provides a genetic tool for grape breeders and geneticists to enhance and accelerate the delivery of improved varieties. The more we learn about grape genetics, fruit composition, and pest and disease resistance, the less screening of intermediate generations we will need to do in the vineyard. Genomic selection will allow breeders to identify the



International interest. Dr. Rudolf Eibach, Julius Kuhn Institut for Grapevine Breeding in Geilweilerhof, Germany (the federal grapevine breeding institute of Germany, with a dwarf grapevine seedling grown from seed sent by Peter Cousins from ‘Pixie’ vines. Dr. Eibach and his colleagues intend to use the grapevines for accelerating breeding and population improvement for disease and pest resistance and to enhance fruit quality.

Photo by Peter Cousins

plants with the desired combinations of alleles and use those plants as parents for the next generation. Speeding up the generations will increasingly be a key part of the improvement of grape breeding.

The next steps in dwarf, rapidly flowering, and precocious germplasm is the development of lines designed for breeding of specific traits and rapid breeding. Working closely with grape growers, wine makers, and researchers will help us prioritize the types of traits that are most important, so that we can introduce dwarf and precocious flowering germplasm to enhance the breeding of new varieties that solve problems and open doors for the grape and wine industry.

Literature cited:

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