In Hawaii, papaya has been genetically engineered to resist ringspot virus: infected plants on (left), virus-resistant (right).

requirements might be necessary if commercialization of these crops is to become a reality (Miller and Bradford 2010; Mou and Scorza 2010). In the survey, conducted between 2005 and 2008, research publications identified 78 different specialty crops and more than 250 traits; however, none of the crops had received complete regulatory approval or been commercialized (Miller and Bradford 2010).

While the lengthy regulatory approval process may account for some of these delays and market failures, public approval and consumer and export-market acceptance will remain the ultimate hurdles in the marketplace success of genetically engineered specialty crops (Astrid 2009; Huffman and Rousu 2006; Lusk et al. 2004). The degree of market acceptance varies, with some markets being more affected than others by international attitudes about genetic engineering.

Transgrafting presents a potential way to address consumer acceptance issues and allow the fruit and nut tree industries to realize some of the possible benefits of genetic engineering technology. To move transgrafting technologies toward implementation efficiently and effectively, scientists and legislators must establish clear lines of communication and create supportive regulatory frameworks. Moreover, industry backing will be paramount given the long time frames and costs related to genetic engineering. Ultimately, however, consumer education and attitudes toward transgrafting will be a pivotal aspect. It is important that all of these factors are addressed if specialty crops, such as fruit and nut trees, are to profit from the benefits biotechnology can provide.

V.M. Haroldsen is Scientific Analyst, Morrison and Foerster, San Francisco; G. Paulino is Manager of Business Development, SPRIM, San Francisco; C.L. Chi-Ham is Director of Biotechnology Resources, PIPRA, Davis; and A.B. Bennett is Professor, Department of Plant Sciences, UC Davis, and Executive Director, PIPRA.

A Plant Sciences Departmental GSR award and the Rockefeller Foundation supported work performed at UC Davis. Specific information on individual fruit and nut crops is available from the authors upon request.

Regulatory status of transgrafted plants is unclear

by Victor M. Haroldsen, Gabriel Paulino, Cecilia L. Chi-Ham *and* Alan B. Bennett

The regulatory implications of using transgrafted plants are currently unknown. A plant's vascular system can selectively transport across graft junctions endogenous elements such as full-length RNAs, sRNAs, proteins, hormones, metabolites and vitamins, and even elicit epigenetic effects, heritably changing the way genes are expressed without changing the actual DNA sequence. However, not all of these elements are transported freely, and they either require specific molecular signals or cellular transporters to aid in their movement through a plant's vascular system.

These transfers are understood to a degree (Haroldsen et al. 2012), but what is less clear is how the movement of these elements from transgenic rootstocks to scions might affect the regulatory approval process for a transgrafted plant — a product developed using transgenic tools and yet not containing transgenic DNA in the scion product. It cannot be said with certainty if transgenic RNAs, sRNAs or proteins produced in rootstocks may make their way to the nontransgenic scion. Furthermore, some of these elements may have short half-lives, making it difficult to determine by testing whether the final crop was produced using a transgraft.

There is no precedent within the regulatory framework coordinated by the U.S. Department of Agriculture, U.S. Food and Drug Administration and U.S. Environmental Protection Agency regarding how a transgrafted, genetically engineered rootstock and wild-type scion might be regulated. U.S. regulation identifies genetically engineered crops through a product-based policy; that is, if the final product contains transgenic material, then it is considered genetically engineered. However, even if scions are shown to be free of transgenic DNA, since transgrafted crop products are new to consumption, it is likely that safety assessments will be required prior to their market release. They would potentially, however, be classified as a conventional and not genetically modified food in the United States.

Conversely, in the European Union, if biotechnology tools are used in the process of developing a crop, then they fall under EU legislation for genetically engineered crops. In this case, regardless of whether the final transgrafted crop product contains transgenic material (DNA, RNA or proteins) or not, it would be classified as genetically modified. For example, German authorities decided in 2010 that any grapes or wine produced from transgenic rootstocks must be labeled as genetically engineered (Heselmans 2011).

This international policy difference will put EU regulators in a difficult situation in the future, when importing crops harvested from transgrafted plants produced in the United States. How will they identify a nontransgenic crop product that has been developed using transgenic tools? How can they be certain that crops imported from countries such as the United States are not genetically engineered (by EU

References

Aguero CB, Uratsu SL, Greve C, et al. 2005. Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Mol Plant Pathol 6(1):43–51.

Astrid D. 2009. The dispersion and development of consumer preferences for genetically modified food — a meta-analysis. Ecol Econ 68(8–9):2182–92.

Atkinson RC, Beachy RN, Conway G, et al. 2003. Intellectual property rights. Public sector collaboration for agricultural IP management. Science 301(5630):174–5.

Bernard JC, Gifford K, Santora K, Bernard DJ. 2009. Willingness to pay for foods with varying production traits and levels of genetically modified content. J Food Distribution Res 40(2):1–11.

[CDFA] California Department of Food and Agriculture. 2007. Pierce's Disease/Glassy Winged Sharpshooter Research Scientific Review Final Report, August. Pierce's Disease Research Scientific Advisory Panel.

COGEM. 2006. New Techniques in Biotechnology. COGEM Report CGM/061024-02. www.cogem.net/index.cfm/en/publications/publicatie/new-techniques-in-plant-biotechnology (accessed Oct. 21, 2011).

Escobar MA, Civerolo EL, Summerfelt KR, Dandekar AM. 2001. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. PNAS USA 98(23):13437–42.Escobar MA, Leslie CA, McGranahan GH, Dandekar AM. 2002. Silencing crown gall disease in walnut (*Juglans regia* L.). Plant Sci 163(3):591–7.

Gonsalves D. 2004. Virus-resistant transgenic papaya helps save Hawaiian industry. Cal Ag 58(2):92–3.

Graff GD, Wright BD, Bennett AB, Zilberman D. 2004. Access to intellectual property is a major obstacle to developing transgenic horticultural crops. Cal Ag 58(2):120–6.

Haroldsen V, Szczerba MW, Aktas H, et al. 2012. Mobility of transgenic nucleic acids and proteins within grafted rootstocks for agricultural improvement. Frontiers Plant Sci 3:39.

Heselmans M. 2011. Legal uncertainties obstruct breeders. Prophyta 52:16–21.

Huffman W, Rousu M. 2006. Consumer attitudes and market resistance to biotech products. In: Just RE, Alston JM, Zilberman D (eds.). *Regulating Agricultural Biotechnology: Economics and Policy*. New York, NY: Springer Sci Bus Media. p 201–25.

James C. 2010. Global Status of Commercialized Biotech/GM Crops: 2010. ISAAA Brief No 42. International Service for the Acquisition of Agri-Biotech Applications. Ithaca, NY.

Kalaitzandonakes N, Alston JM, Bradford KJ. 2006. Compliance costs for regulatory approval of new biotech crops. In: Just RE, Alston JM, Zilberman D (eds.). *Regulating Agricultural Biotechnology: Economics and Policy*. New York, NY: Springer Sci Bus Media. p 37–57.

Lev-Yadun S, Sederoff R. 2001. Grafting for transgene containment. Nat Biotech 19(12):1104.

Lusk JL, House LO, Valli C, et al. 2004. Effect of information about benefits of biotechnology on consumer acceptance of genetically modified food: Evidence from experimental auctions in the United States, England, and France. Eur Rev Agric Econ 31(2):179–204.

Miller JK, Bradford KJ. 2010. The regulatory bottleneck for biotech specialty crops. Nat Biotechnol 28(10):1012–4.

Mou B, Scorza R (eds.). 2010. *Transgenic Horticultural Crops: Challenges and Opportunities.* Oxon, Eng.: Taylor Francis. 354 p.

Mulvaney DR, Krupnik TJ, Koffler KB. 2011. Transgenic rice evaluated for risks to marketability. Cal Ag 65(3):161–7.

Oerke EC, Dehne HW. 2004. Safeguarding production — losses in major crops and the role of crop protection. Crop Prot 23(4):275–85.

[USDA APHIS] US Department of Agriculture Animal and Plant Health Inspection Service. 2010. USDA Announces Quarantine to Prevent Spread of Citrus Disease. www.aphis.usda.gov/newsroom/content/2010/06/citrus_disease.shtml (accessed Jan. 4, 2012).

USDA APHIS. 2011. Biotechnology Permits, Notifications, and Petitions. Biotechnology Regulatory Services. www.aphis.usda.gov/biotechnology/brs_main.shtml (accessed Mar. 15, 2011).

[USDA ERS] USDA Economic Research Service. 2011. Value of Production for Selected Fruit and Tree Nuts, by State, 2007 to Date. www.ers.usda.gov/publications/fts/Yearbook11/FTS2011.pdf (accessed Jan. 4, 2012).

[USDA NASS] USDA National Agricultural Statistics Service. 2011. Crop Value Summary 2011. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1050 (accessed Feb. 4, 2012).

definitions) when testing may not differentiate between conventionally grown crops and those from transgrafted plants? While the European Union may elect to implement process monitoring of new technologies to ensure proper labeling, documenting every step of the production process and tracking the final products of transgrafted crops, it would be difficult to guarantee the genetically engineered status of imports from outside the European Union.

To address this difficulty, and assuming tests can be developed that easily and robustly detect the presence or absence of transgene elements in the final crop, a threshold limitation could be established. The EU threshold for allowable levels of "adventitious mixing" of genetically engineered seed into conventional seed is 0.9%. The expectation for transgrafted crops, in particular first-generation transgrafts (see page 67), is that any transgenic DNA, mRNA, siRNA or protein would fall below the 0.9% level. While current EU legislation would likely need to be revised, it is possible that EU regulators would allow transgrafted fruit or nut products to enter the European Union, so long as transgenic material was below the 0.9% threshold. While strictly conjecture, at the least, this sort of threshold limitation should be included in discussions of alternatives to current regulatory requirements.

In the United States, transgrafting applications will likely be dealt with on a case-by-case basis as they are introduced into the regulatory process (C. Wood, USDA Biotechnology Regulatory Services, personal communication, September 2010). This would be in line with suggestions by the Dutch scientific advisory committee on genetically modified organisms (COGEM 2006). In anticipation of regulatory scrutiny, it will be important for scientists to gather experimental information determining to what degree transgenic elements move across the graft junction in different plant species and different types of coding and noncoding genetic constructs.

Unlike plant model systems, such as *Arabidopsis* sp., analyses of genetic material from fruit and nut crops tend to be compounded by high levels of phenolic compounds, polysaccharides and other secondary metabolites. Nevertheless, laboratory experiments have been carried out in walnut, grape and tomato regarding the mobility of transgenic elements, and the results are in the process of being published. This information should assist regulatory bodies in determining what portion of the scion, if any, should be regulated.

To illustrate these issues, imagine that a transgrafted orange is developed with transgenic siRNA in the rootstock that wards off nematodes. Tests on the scion leaf material do not reveal the presence of siRNA, but when the fruit is tested transgenic siRNA is detected. However, tests also show that after the oranges are harvested, the transgenic siRNA decreases over a short time to nondetectable levels. In the United States, after regulatory approval the oranges would not be required to be labeled as genetically engineered. If these sames oranges were exported to the European Union, siRNA would be undetectable in tests regardless of the transgraft, but under EU legislation they would be classified and labeled as genetically engineered. So unless the U.S. seller directly informs the importer that the oranges were grown with a transgraft, they would have no way of knowing since the siRNA is undetectable after picking. This example highlights the difficulties arising from policy differences, which could hamper the future commercialization of transgrafting technologies currently in the developmental pipeline.