can result in clinically safe mAbs, as in the case of trastuzumab, the small proportion of murine sequences in the CDRs that can remain may still be sufficient to induce immunogenicity. Such is the case reported for natalizumab, a humanized mAb of murine origin prescribed for the treatment of multiple sclerosis. Antinatalizumab antibodies with affinity to the CDR regions of natalizumab were discovered in the serum of treated patients, and the levels of circulating natalizumab were depleted (Subramanyam, 2008).

The second potential immunogenic property of mAbs is their glycosylation profile. Plant-produced mAbs bearing nonmammalian *N*-glycans, β1,2-xylose, and α1,3-fucose shown in Figure 17.2A, may be capable of eliciting an immune response in human patients, as these two glymodifications have been reported to be immunogenic in various laboratory animals, including mice and rats (Bardor et al., 2003), rabbits (Faye et al., 1993), and goats (Kurosaka et al., 1991). In all instances, the animals were immunized with horseradish peroxidase, which is heavily glycosylated with both β1,2-xylose and α1,3-fucose, and antibodies with affinity to both sugars were isolated from these immune sera (Bardor et al., 2003; Faye et al., 1993; Kurosaka et al., 1991). Despite the strong evidence of the immunogenicity of β1,2-xylose and α1,3-fucose in mammals, little evidence of their immunogenicity has been reported in humans, although their allergenicity has been well established for more than a decade (Altmann, 2007). Anti-xylose and anti-fucose IgEs, collected from patients with pollen allergies, were found to show affinity toward plant-produced 2G12, which was xylosylated and α1,3-fucosylated, suggesting that patients with pollen allergies may develop an allergic reaction to plant-glycosylated mAbs. However, whether the same reaction would be mounted in nonallergic patients is unclear (Jin et al., 2008). To further complicate matters, Elelyso is both α1,2-xylosylated and α1,3-fucosylated, and was approved for human use by the FDA, suggesting that the presence of these two sugars may not pose a significant immunogenic risk in humans (Shaaltiel et al., 2007).

Although the long-term impact of plant-specific xylose and fucose in plant-produced biopharmaceuticals is unknown, the elimination of these two sugars from the glycan profiles of therapeutic mAbs is a priority for therapeutic mAb producers and regulatory agencies (Bosch et al., 2013). The original draft guidance jointly issued by the FDA and the US Department of Agriculture (USDA), agencies that oversee the approval of pharmaceuticals and their safety, explicitly states that plant-produced therapeutics should be screened for potentially immunogenic xylose and allergenic plant-specific *N*-glycans (FDA, 2002).

17.4.5 Need to Humanize Glycosylation in Plants

The development of biosimilar drugs in plants requires a humanized *N*-glycosylation system. To achieve a CHO-like *N*-glycosylation, for example, xylosylation and α1,3-fucosylation must first be eliminated, as shown in Figure 17.2B, and β1,4-galactosylation must be introduced as shown in Figure 17.2C.

It is worthy to note that the FDA and USDA classify xylose as only *potentially* immunogenic and do not explicitly require the complete elimination of xylose and plant-specific *N*-glycans (USFDA, USDA, 2002). However, to minimize all possible