WOZNIAK, CHRIS A.[1], and LOWELL D. OWENS[2], [1]USDA, Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND 58105, and [2]USDA, Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD 20705. Tungsten-mediated hydrolysis of β-glucuronide substrates following microprojectile bombardment.

Metal microcarriers of gold, silicon carbide and tungsten are used in microprojectile bombardment for the introduction of DNA into plant cells. During the development of a transformation protocol for sugarbeets, it was noted that a blue precipitate was formed following the use of tungsten microcarriers in the absence of *gusA* DNA, which encodes β-glucuronidase (GUS). Further evaluation indicated that tungsten microspheres were capable of catalyzing the hydrolysis of X-gluc, salmon X-gluc, and magenta X-gluc, the histochemical substrates used for detection of GUS. Tungsten microspheres accelerated into sugarbeet cells resulted in a blue precipitate when X-gluc assays were prolonged ($>$24 hours) and gave rise to blue-stained cells. The fluorogenic substrate 4-methylumbelliferyl β-D-glucuronidase (MUG) was similarly hydrolyzed in the presence of tungsten microspheres in the absence of DNA. Gold microspheres and silicon carbide fibrils did not result in hydrolysis of any of the β-glucuronide substrates tested. Incubation of MUG with millimolar concentrations of Cu^{2+}, Fe^{2+} and Zn^{2+} also resulted in hydrolysis. Heat and protease treatments of tungsten microcarriers, along with standard microbiological analysis, ruled out the presence of contaminating proteins and microbes, respectively. Attention to the use of tungsten microcarriers, metal ions of Cu, Fe and Zn at millimolar concentrations, and the length of incubation during histochemical assays is indicated. The use of DNA-minus and microcarrier-minus controls is recommended when using tungsten microspheres.