Letters

Scientific data published by a peer-reviewed journal should be properly interpreted: a reply to the letter by Gressel *et al.* (2014)

Gressel *et al.* (2014; in this issue of *New Phytologist*, pp. 360–362) disapprove of media coverage of our recent peer-reviewed paper (Wang *et al.*, 2014; this issue of *New Phytologist*, pp. 679–683), and they question whether the paper can be seen as 'deserving publication'. In this study, our main hypothesis was that overproduction of a key metabolic enzyme (EPSPS) may have the unanticipated effect of stimulating plant growth and fecundity in crop–weed hybrids of rice (*Oryza sativa*). This enzyme is the target of glyphosate-based herbicides, which are widely used on transgenic glyphosate-tolerant crops. We found that transgenic overexpression of an endogenous *epsps* gene from rice – which was developed to confer glyphosate resistance – was associated with increases in EPSPS protein levels, tryptophan concentrations, photosynthetic rates, seed germination, plant growth and fecundity of crop-weed rice progeny, relative to their nontransgenic counterparts. This transgenic event was crossed into four weedy rice accessions and tested under field conditions in China.

Here, we address several of our critics' questions and concerns, while noting that others fall outside the scope of our research and therefore are not relevant to the publication. Given the inherent constraints of conducting ecological field studies with strictly regulated, experimental transgenes, we argue that our paper is similar in scope and depth to many other publications in this field. However, in an effort to provide a concise summary of the work, we left out some important details that are included below. We appreciate the opportunity to address these gaps in the following section.

How was the glyphosate-resistant transgenic line that overexpressed *epsps* developed?

Gressel *et al.* argued that we did not provide sufficient details about the transgenic construct in our parental EP3 transgenic rice line, making it difficult to evaluate or reproduce our study, nor did we explain how its glyphosate resistance was documented. This was an oversight on our part. Su *et al.* (2008), which is published in Chinese and which describes several experimental transgenic events, showed that the EP3 transgenic event has one copy of the *epsps* transgene driven by an ubiquitin promoter from maize and is resistant to glyphosate, as intended. Fig. 1 shows the major components of this construct. Briefly, Su *et al.* obtained the EP3 line using an endogenous *epsps* gene from Minghui-63 rice that was described in Xu *et al.* (2002). Without introns, the endogenous *epsps* gene is 1585 bp (NCBI, http://www.ncbi.nlm.nih.gov/nuccore/15778435), while the full length of the *epsps* sequence with introns is 3661 bp (NCBI, http://www.ncbi.nlm.nih.gov/nuccore/15724391). The transgenic EP3 used the *epsps* sequence without introns (indicated in Su *et al.*, 2008). Others could repeat our experiment by creating rice lines similar to EP3, using sequence data in NCBI and crossing these rice lines with accessions of weedy rice. In Wang *et al.*, we used specific PCR primers for discriminating between sequences from the endogenous *epsps* and the smaller inserted transgenic *epsps*, with fragments that were *c.* 1000 and 700 bp, respectively.

Su *et al.* (2008) produced 84 independent clones and used PCR amplification to confirm the presence of the target *epsps* transgene in the T_1 EP3 generation. T_1 and T_2 progeny obtained from selfing were grown under field conditions and sprayed with glyphosate (Roundup) at commercially recommended dosages to confirm glyphosate resistance (note: the English abstract was translated incorrectly, using the word glufosinate in place of glyphosate in the third sentence). This dosage of Roundup was described as 3 l ha^{-1}. The commercial Roundup formulation was 41% glyphosate isopropylamine salt (molecular formula: $C_3H_9N{\cdot}C_3H_8NO_5P$; molecular weight: 228.2), which is equal to 30% glyphosate (molecular formula: $C_3H_8NO_5P$; molecular weight: 169.1). For the dilution of Roundup before spraying, it is recommended to use 3–6 l ha^{-1} of Roundup solution diluted with water (450 l ha^{-1}) in rice fields, which is equivalent to 11.83–23.66 mM of glyphosate for the concentration.

T_3 plants were used for Southern blot analysis and further glyphosate screening (Su *et al.*, 2008). When the T_3 EP3 progeny were tested at the three-leaf stage and compared to nontransgenic, Minghui-86 controls, 100% of the EP3 plants survived from treatments with 15 mM glyphosate (Sigma), which killed all of the control plants (Table 1). The T_3 EP3 plants were not tested for glyphosate resistance under field conditions by Su *et al.* (2008), as

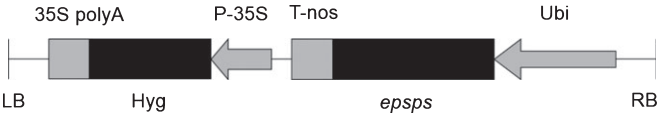

Fig. 1 Major components of the *epsps* transgene construct of the pCUEP plasmid (with the transgenes inserted into the pCAMBIAl300 backbone) for the EP3 rice (*Oryza sativa*) line. LB, the left border of the construct; 35S polyA, the terminator of the hygromycin selectable marker gene (Hyg) driven by a cauliflower mosaic virus (CaMV) 35S promoter (P-35S); T-nos, terminator of *epsps* transgene driven by an ubiquitin promoter from maize; RB, right border of the construct. The *epsps* transgene is modified from the endogenous gene of Minghui-63 rice. The *epsps* transgene sequence without introns that was used to produce this EP3 line can be found at: http://www.ncbi.nlm.nih.gov/nuccore/15778435. See text and Su *et al.* (2008) for further details.

New Phytologist

was the case for the T_1 and T_2 generations, but these plants were resistant to concentrations that were lethal to the nontransgenic controls (Table 1; Fig. 2) and were selfed to obtain the T_4 and T_5 generations. This implies that it is accurate to describe the T_5 EP3 plants as being resistant to glyphosate in Wang *et al.*

Gressel *et al.*'s interpretation of our findings

Before accepting Gressel *et al.*'s critique, we encourage readers to study our publication and reflect on the content, nuances and caveats that cannot be captured in media reports or letters to the Editor. Also, it is important to distinguish between our interpretation of the data and the interpretation of our data by others, including the opinions mentioned in *Nature* (Qiu, 2013) and those of Gressel *et al.* Below, we discuss four major issues related to Gressel *et al.*'s interpretation of our original paper.

Position effects

As with any study of a single transgenic event, we noted that genetically engineered (GE) plants and their nonGE counterparts differed in the presence or absence of the inserted construct, 'as well as the selectable marker gene and any crop-specific genes that may be linked to the transgene insertion site'. Position effects and other artifacts associated with tissue culture can alter a plant's phenotype (e.g. Matzke & Matzke, 1998; Bhat & Srinivasan, 2002; Zeller *et al.*, 2010). Therefore, it is well known that studying a single transgenic event does not allow one to generalize about all events with a particular transgene, but this caveat is rarely explained when results are simplified in the news media. Unfortunately, options for obtaining more than one transgenic event for research purposes often are not available (e.g. Dalton, 2002; Burke & Rieseberg, 2003; Sasu *et al.*, 2009). In our case, the cost of developing and breeding several transgenic events into multiple weedy rice accessions led us to focus on a single transgenic event. Contrary to the implications of Gressel *et al.,* our methods and interpretations regarding fitness-related effects of the *epsps* transgene are similar in approach to many previous ecological publications on this topic (e.g. Stewart *et al.*, 1997; Snow *et al.*, 1999, 2003; Burke & Rieseberg, 2003; Vacher *et al.*, 2004; Chen *et al.*, 2006; Guadagnuolo *et al.*, 2006; Laughlin *et al.*, 2009; Sasu *et al.*, 2009; Londo *et al.*, 2011; Xia *et al.*, 2011; Yang *et al.*, 2011, 2012).

Table 1 Mean percentage survival of EP3 (T_3) and Minghui-86 rice (*Oryza sativa*) seedlings under different concentrations of glyphosate (Sigma) at the three-leaf stage

	Glyphosate concentration (mM)					
Rice line	0	2	5	10	15	20
EP3 (GE)	100	100	100	100	100	0
Minghui-86	100	100	27.0	7.1	0	0

$N = 3$ replicates with five plants per replicate. From Su *et al.* (2008).

Fig. 2 Seedlings of T_3 EP3 transgenic rice (*Oryza sativa*) (left) and its nontransgenic parent Minghui-86 rice (right) after spraying with 15 mM of glyphosate (Sigma), as described in Su *et al.* (2008). Photo provided by Professor Jun Su, Fujian Academy of Agricultural Sciences (China).

Fitness-related traits

Gressel *et al.* argue that our fitness study was 'flawed' and 'highly artificial'. We found that F_2 GE crop–weed hybrids produced 48–125% more seeds per plant than their nonGE counterparts in common garden field experiments. This suggests that the GE trait could persist and increase in frequency over time, with the caveat that 'we do not know how other stages of the life cycle, such as seed longevity or seedling establishment, are affected by the *epsps* transgene'. We also noted that 'further life-cycle ecological research is needed to examine whether weedy rice biotypes that overproduce EPSPS could have accelerated population growth rates and enhanced competitive ability relative to existing biotypes in the absence of glyphosate, as we surmise'. Many studies showing fitness-related effects of specific transgenes have used similar experimental approaches with similar caveats (see examples cited above). Common garden field experiments are widely used in other areas of ecology to test for differences in fitness-related traits between groups of interest, such as hybrids vs parents, selfed vs outcrossed progeny, native vs introduced biotypes, or resistant vs susceptible biotypes. Although these conditions may be described as 'artificial', common garden experiments represent an important tool for evolutionary ecologists.

Competition

Gressel *et al.* incorrectly imply that we intended to test for differences in 'relative competitive ability' between GE and nonGE crop–weed hybrids. This would have required a much more elaborate experimental design. Gressel *et al.* introduce the term 'competitive fitness' (not used in our paper) and proceed to discuss self-thinning and the design of competition experiments at length. However, our study was not intended to examine competitive ability *per se*. We explicitly stated that 'the mixed planting treatment was included to increase the likelihood of detecting small fitness differences that might be evident only with direct competition between GE plants and their non-GE counterparts'. In fact, these differences were more pronounced in the mixed planting treatment, as expected.

Biosafety and regulatory implications

Contrary to what is implied by Gressel *et al.*, our paper does not mention biosafety, nor do we recommend how the *epsps* trait or other transgenic traits should be regulated. A much broader topic that Gressel *et al.* bring up is whether fitness-related transgenic traits are sufficiently different from other crop traits to require any regulation at all. We did not discuss these topics in our paper. Instead, we noted that fitness-enhancing transgenes could allow novel traits to persist in wild or weedy populations that hybridize with the crop. This type of information about gene flow and its consequences is a standard requirement of many regulatory agencies dealing with GE crops (but see Ledford, 2013). Echoing a common theme of research articles about gene flow from GE crops, Stewart *et al.* (1997) argued that when a transgene is associated with increased fecundity and suitable habitat is available, 'there is a likelihood of enhanced ecological risk associated with the release of certain transgene/crop combinations'.

Scientists and the media

Regarding best practices for scientists who publish research on politically or ethically controversial topics, including genetically engineered crops, we agree that it is important to be as patient, accurate and strategically sensitive as possible. Peer-reviewed journals allow one to do this with careful editing and feedback from reviewers. By contrast, authors have very limited control, if any, over how such findings will be interpreted by science writers or the popular press. In the face of nearly instantaneous internet connectivity, there is no way for scientists to revise or correct inappropriate statements in the media, other than to follow up with further information and clarification. Does this mean that new and interesting findings should be left unpublished or, if published, that the authors should not participate in interviews? We think not.

As scientists, we feel that we have an obligation to discuss our findings with both scientific and public audiences in a timely and responsible manner. We endeavored to write our paper as clearly as possible, without undue speculation, and fully expecting that we and others will pursue studies to confirm or reject our hypothesis in the future. A press release from Ohio State University included an interview with Allison Snow, who explained that: 'We don't know yet if our findings are going to be generalizable…' and 'ecological studies such as ours can help inform risk assessment and biosafety oversight'. Gressel *et al.* give the impression that we misled journalists as to the rigor and implications of our study, but this is untrue. Our paper met standard criteria for peer-reviewed publications in evolutionary ecology, and it was subjected to peer review.

Conflict of interest

The authors declare no financial interest in the use of herbicides nor in genetically engineered (GE) herbicide-resistant plants.

Acknowledgements

We thank Professor Jun Su for clarifications regarding the EP3 transgenic line and the colour photo for Fig. 2.

Bao-Rong Lu[1]*, Allison A. Snow[2], Xiao Yang[1] and Wei Wang[1]

[1]Ministry of Education, Key Laboratory for Biodiversity Science and Ecological Engineering, Department of Ecology and Evolutionary Biology, Fudan University, Handan Road 220, Shanghai 200433, China;
[2]Department of Evolution, Ecology & Organismal Biology, Ohio State University, Columbus, OH, USA
(*Author for correspondence: tel +86 21 65643668;
email brlu@fudan.edu.cn)

References

Bhat SR, Srinivasan S. 2002. Molecular and genetic analyses of transgenic plants: considerations and approaches. *Plant Science* **164**: 673–681.

Burke JM, Rieseberg LH. 2003. Fitness effects of transgenic disease resistance in sunflowers. *Science* **300**: 1250.

Chen LY, Snow AA, Wang F, Lu B-R. 2006. Effects of insect-resistance transgenes on fecundity in rice (*Oryza sativa*): a test for underlying costs. *American Journal of Botany* **93**: 94–101.

Dalton R. 2002. Superweed study falters as seed firms deny access to transgenes. *Nature* **419**: 655.

Gressel J, Stewart CN Jr, Giddings LV, Fischer AJ, Streibig JC, Burgos NR, Trewavas A, Merotto A Jr, Leaver CJ, Amman K *et al.* 2014. Overexpression of *epsps* transgene in weedy rice: insufficient evidence to support speculations about biosafety. *New Phytologist.* **202**: 360–362.

Guadagnuolo R, Clegg J, Ellstrand NC. 2006. Relative fitness of transgenic *vs.* non-transgenic maize × teosinte hybrids: a field evaluation. *Ecological Applications* **16**: 1967–1974.

Laughlin KD, Power AG, Snow AA, Spencer LJ. 2009. Risk assessment of genetically engineered crops: fitness effects of virus-resistant transgenes in wild *Cucurbita pepo. Ecological Applications* **19**: 1019–1101.

Ledford H. 2013. US regulation misses some GM crops. *Nature* **500**: 389–390.

Londo JP, Bollman MA, Sager CL, Lee EH, Watrud LS. 2011. Changes in fitness-associated traits due to the stacking of transgenic glyphosate resistance and insect resistance in *Brassica napus* L. *Heredity* **107**: 328–337.

Matzke AJM, Matzke MA. 1998. Position effects and epigenetic silencing of plant transgenes. *Current Opinion in Plant Biology* **1**: 142–148.

Qiu J. 2013. Genetically modified crops pass benefits to weeds: herbicide resistance and other genetic modifications could confer an advantage on plants in the wild. *Nature* **500**: 389–390.

Sasu MA, Ferrari MJ, Du D, Winsor JA, Stephenson AG. 2009. Indirect costs of a nontarget pathogen mitigate the direct benefits of a virus-resistant transgene in wild *Cucurbita. Proceedings of the National Academy of Sciences, USA* **106**: 19067–19071.

Snow AA, Andersen B, Jørgensen RB. 1999. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *Brassica rapa. Molecular Ecology* **8**: 605–615.

Snow AA, Pilson D, Rieseberg LH, Paulsen M, Pleskac N, Reagon MR, Wolf DE, Selbo SM. 2003. A *Bt* transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications* **13**: 279–286.

Stewart CN, All JN, Raymer PL, Ramachandran S. 1997. Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Molecular Ecology* **6**: 773–779.

Su J, Chen GM, Tian DG, Zhu Z, Wang F. 2008. A gene encodes 5-enolpyruvylshikimate-3-phosphate mutagenized by error-prone PCR conferred rice with high glyphosate-tolerance. *Molecular Plant Breeding* **6**:

830–836. (Chinese text with English abstract. [WWW document] URL http://www.cnki.com.cn/Article/CJFDTotal-FZZW200805005.htm) [accessed 9 January 2014].

Vacher C, Weis AE, Hermann D, Kossler T, Young C, Hochberg ME. 2004. Impact of ecological factors on the initial invasion of *Bt* transgenes into wild populations of birdseed rape (*Brassica rapa*). *Theoretical and Applied Genetics* **109**: 806–814.

Wang W, Xia H, Yang X, Xu T, Si HJ, Cai XX, Wang F, Su J, Snow AA, Lu B-R. 2014. A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (*Oryza sativa*) without herbicide. *New Phytologist* **202**: 679–683.

Xia H, Lu B-R, Xu K, Wang W, Yang X, Yang C, Luo J, Lai F, Ye W, Fu Q. 2011. Enhanced yield performance of *Bt* rice under target-insect attacks: implications for field insect management. *Transgenic Research* **20**: 655–664.

Xu J, Feng D, Li X, Chang T, Zhu Z. 2002. Cloning of genomic DNA of rice 5-enolpyruvylshikimate 3-phosphate synthase gene and chromosomal localization of the gene. *Science in China (Series C)* **45**: 251–259.

Yang X, Wang F, Su J, Lu B-R. 2012. Limited fitness advantages of crop-weed hybrid progeny containing insect-resistant transgenes (*Bt/CpTI*) in transgenic rice field. *PLoS ONE* **7**: e41220.

Yang X, Xia H, Wang W, Wang F, Su J, Snow AA, Lu B-R. 2011. Transgenes for insect resistance reduce herbivory and enhance fecundity in advanced generations of crop-weed hybrids of rice. *Evolutionary Applications* **4**: 672–684.

Zeller SL, Kalinina O, Brunner S, Keller B, Schmid B. 2010. Transgene × environment interactions in genetically modified wheat. *PLoS ONE* **5**: e11405.

Key words: biosafety, EPSPS, fitness, genetically engineered plant, herbicide resistance, *Oryza sativa*, position effect, weedy rice.