JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2007,** *55,* 1645−1647 **1645**

Rebuttal on Results from the FATEALLCHEM Project

Sir: We thank Dr. Aki Sinkkonen for his interest in our work, presented in Fomsgaard (*1*), Macías et al. (*2*), and Mathiassen et al. (*3*). We appreciate Dr. Sinkkonen's recognition of the importance of the FATEALLCHEM project and the value of our research in the field of allelopathy.

Dr. Sinkkonen opens his comments expressing his disagreement "with one of the major conclusions in the FATEALLCHEM ... that future research should include the utilization and development of varieties with maximum production of benzoxazinones". We stress that in all three papers we have used expressions such as "optimal" or "increased" or "enhanced" in relation to production of benzoxazinones. The word "maximum" was used only once. We realize that the use of the word "maximum" can be misunderstood. We therefore stress that the project results showed that the levels of benzoxazinoids found in wheat varieties that are cultivated nowadays around the world are not high enough if formation of the more active metabolites in soil is sought. This is due to the fact that transformation pathways differ, depending on the initial concentrations. The most active metabolite, 2-aminophenoxazin-3-one (APO), can suppress a number of weeds and does not cause any harm to the investigated beneficials, that is, worse than the harm pesticides cause. For the production of APO in the soil environment to take place, higher concentrations of the benzoxazinones are needed. Our detailed comments to Dr. Sinkkonen follow below.

First, we agree with Dr. Sinkkonen that in some cases soil microorganisms may acclimate rapidly to metabolize allelochemicals that occur in soil at high concentrations and that soil bacteria also can adapt genetically to the presence of xenobiotics. Whether soil bacteria can adapt to the presence of, for instance, pesticides depends on the molecular structure of the compounds as shown in numerous publications on the fate of pesticides that were published during the past decades. If microorganisms adapt to higher concentrations of some allelochemicals, this could certainly affect the allelopathic phenomenon. Dr. Sinkkonen suggests this phenomenon would diminish the efficiency of natural phytotoxins in weed control. In the case of benzoxazinones, however, our numerous papers on soil degradation and efficiency studies on the transformation products have revealed that the microbial degradation of benzoxazinones provided several chemicals with much more powerful phytotoxicity and stability (*2*, *4*−*10*). The major part of the degradation studies in the project were performed quantitatively, and even if some of the studies in natural conditions revealed that only part of the parent compound was converted to the more biologically active compound [as in the case of 2-benzoxazolinone (BOA) transformation to APO, for instance], an overall higher biological activity was obtained, due to the high activity of APO.

Second, current crop varieties can be used to estimate the effects of the allelochemicals they release on target organisms. The fact that they produce benzoxazinones make them possible candidates for modeling the effects of potential crops with an enhanced capacitiy to produce benzoxazinones. We point out that in the degradation studies by Marías et al. (*4*) we used soil in which the wheat varieties previously had been cultivated, and consequently the microbes able to transform benzoxazinones were present. The degradation studies are directed toward the modeling of allelopathic interactions: The amount and rhythms of allelochemical production by the donor plant, the degradation rates and the chemicals produced by those processes, the capacity of the target weed to take up those chemicals, and the phytotoxic effects they have after the uptake process can be assessed. It is also possible to perform a qualitative (which chemicals) and quantitative (what amounts of each chemical) analysis throughout the whole process. Our latest research completely characterized those interactions. Those results will be reported shortly, but an advance publication of that research can be found at Macías et al. (*11*, *12*).

The target species in the study of Mathiassen et al. (*3*) were selected to represent common monocot and dicot weed species in Europe. The focus of this study was to examine the potential of weed suppression by green manure of wheat and not to explain differences in weed susceptibility by an evolutionary theory. With regard to the target species we chose for the structure−activity relationship (SAR) studies in Macías et al. (*2*), we state the following: Our development of benzoxazinones in the search for natural herbicide models has a fundamentally practical purpose: *Lolium rigidum* and *Avena fatua* are no. 1 and 2, respectively, in the worst weeds classification that can be found on the International Survey of Herbicide Resistant Weeds, hosted by the Weed Science Society of America (*13*), among others. Several weeds mentioned in the report by Schulz et al. (*14*) belong to the Poaceae family; they evolved together with other Poaceae plants and probably grow vigorously in the presence of benzoxazinones, but those were *natural* benzoxazinones. When we introduce synthetic modifications, the problem becomes much more complicated to analyze in light of co-evolutionary theory. Some Poaceae weeds resist natural benzoxazinones, including the ones we tested. This could be due to a detoxification mechanism acquired by co-evolution with benzoxazinone producing plants. Our results point clearly in that direction, but the benzoxazinones we are proposing as herbicide models are not the natural ones; they are synthetic models. The synthetic modifications we performed do empower their phytotoxicity and wheat/wild oat selectivity. This can be seen very clearly in our most recent research on natural herbicide models based on benzoxazinones (*15*, *16*), in addition to our paper (*2*) commented on by Dr. Sinkonnen. The co-evolutionary hypothesis could help to explain the lower effects found for *natural* benzoxazinones in our SAR studies, for both wheat and its weeds. Precisely those effects drove us to discard benzoxazinones with natural-like functionalization for the development of herbicide models.

10.1021/jf062377f CCC: $37.00

In contrast to this, the results obtained for synthetic benzoxazinones oblige us to use the co-evolutionary hypothesis much more carefully. We found that some of the synthetic benzoxazinones we tested affected the Poaceae weeds *L. rigidum* and *A. fatua* to a higher extent than wheat. Thus, detoxification mechanisms for wheat are versatile enough for detoxifiying synthetic benzoxazinones, whereas detoxification mechanisms of *A. fatua* and *L. rigidum* are not that efficient, as very light modifications increase the effects on weeds in a very drastic manner. This could be an explanation in light of the co-evolutionary theory, but we find it very speculative in the absence of concrete detoxification experiments.

What we prefer to do is to perform SAR studies, to relate activity with molecular parameters, and to draw conclusions in light of phytotoxicity results, because we are aiming for phytotoxic compounds. The modifications of those molecular parameters influence both activity and selectivity toward weeds, and that knowledge is used to continue obtaining more active and selective molecules (*15*, *16*). Using natural products as templates for pesticides is a very promising work avenue: allelochemicals have specific interactions with target organisms, different modes of action, and higher biodegradability. However, this research has some associated problems: natural allelochemicals are usually difficult to synthesize, and in many cases they are less phytotoxic than commercial herbicides, because they have to be biosynthesized and preserved inside a plant prior to their release. What we obtained from synthetic benzoxazinones is the following: compounds easy to synthesize, with powerful phytotoxic activity, but still selective (*15*, *16*) and biodegradable (*17*). As all of these statements are confirmed by means of the suitable experiments, we believe our research to be successful and the discussion made in its context to be the least speculative possible.

Wheat is the less affected plant of our SAR studies, and the lower effects found on wheat at the same concentrations that greatly affect other plans illustrate a detoxification capacity. This is true for natural and synthetic benzoxazinones. The origin of this detoxification capacity, as well as the origin of the lack of this capacity for synthetic benzoxazinones in our target organism models, would be a very interesting research field. Results on the mode of action of these phytotoxins (both natural and synthetic) would shed light on this subject, too, and will promote the two-sided research we are conducting: going deeper into the knowledge on the allelopathic phenomena mediated by benzoxazinones and researching the natural herbicide models useful in pest management.

Third, Dr. Sinkkonen points to the fact that benzoxazinones contain nitrogen and that wheat and other cereals may have a tradeoff between benzoxazinone production and growth. He also points to the fact that a maximum production of benzoxazinones may lead to reduced seed losses, and he therefore recommends searching for optimal strategies of production and release and not for maximum production and release. The tradeoff between N in the production of benzoxazinones and N in production of yield is unproven. An analogy can be found in Bt cotton. Metabolic manipulation of the biosynthetic pathway encoding Bt had no significant effect of fiber quality, agronomic characteristics of the cotton, or seed composition (*18*). If such a tradeoff existed, it would not have relevance in all of the cases where we have discussed our results in relation to the use of wheat (or rye) as a cover crop or green manure.

Finally, Dr. Sinkkonen suggests that focus also should be put on the development of cultivars in which the production and release are not stable. Developing plants that exude allelochemicals at irregular intervals during growth does not seem to be realistic at this stage of research. Even if it were realistic, the purpose of optimizing the allelopathic effects of agricultural crops during growth was not the focus of our studies. We emphaszie that the transformation of the released allelochemicals to other compounds with different activities and stabilities in soil is of major concern, because the transformation pathways for benzoxazinones have been shown to be different at different concentration levels. The compounds with enhanced biological activity (for instance, APO) were produced when the benzoxazinones were present in soil at high concentrations. Dr. Sinkkonen suggests that the ecological and evolutionary responses of soil biota to the altered release of phytotoxins must be understood. We can fully agree on that. Several of the FATEALLCHEM papers studied the response of soil and water biota to the presence of benzoxazinones and derivatives (*19*−*24*).

LITERATURE CITED

(1) Fomsgaard, I. S. Chemical ecology in wheat plant−pest interactions. How the use of modern techniques and a multidisciplinary approach can throw new light on a well-known phenomenon: allelopathy. *J. Agric. Food Chem.* **2006**, *54*, 987−990.

(2) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Structure−activity relationship (SAR) studies of benzoxazinones, their degradation products, and analogues. Phytotoxicity on problematic weeds *Avena fatua* L. and *Lolium rigidum* Gaud. *J. Agric. Food Chem.* **2006**, *54*, 1040−1048.

(3) Mathiassen, S. K.; Kudsk, P.; Mogensen, B. B. Herbicidal effects of soil-incorporated wheat. *J. Agric. Food Chem.* **2006**, *54*, 1058−1063.

(4) Macías, F. A.; Oliveros-Bastidas, A.; Marín, D.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Degradation studies on benzoxazinoids. Soil degradation dynamics of (2*R*)-2-*O*-β-D-glucopyranosyl-2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* **2005**, *53*, 554−561.

(5) Fomsgaard, I. S.; Mortensen, A. G.; Idinger, J.; Coja, T.; Blümel, S. Transformation of benzoxazinones and derivatives and microbial activity in the test environment of soil ecotoxicological tests on *Poecilus cupreus* and *Folsomia candida*. *J. Agric. Food Chem.* **2006**, *54*, 1086−1092.

(6) Gents, M. B.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Transformation products of 2-benzoxazolinone (BOA) in soil. *Chemosphere* **2005**, *61*, 74−84.

(7) Understrup, A. G.; Ravnskov, S.; Hansen, H. C. B.; Fomsgaard, I. S. Biotransformation of 2-benzoxazolinone to 2-amino-phenoxazin-3-one and 2-acetylamino-phenoxazin-3-one in soil. *J. Chem. Ecol.* **2005**, *31*, 1205−1222.

(8) Krogh, S.; Mensz, S. J. M.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Fate of benzoxazinone allelochemicals in soil after incorporation of wheat and rye sprouts. *J. Agric. Food Chem.* **2006**, *54*, 1064−1074.

(9) Etzerodt, T.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Elucidating the transformation pattern of the cereal allelochemical 6-methoxy-2-benzoxazolinone (MBOA) and the trideuteriomethoxy analogue [D3]-MBOA in soil. *J. Agric. Food Chem.* **2006**, *54*, 1075−1085.

(10) Jai, C.; Kudsk, P.; Mathiassen, S. K. Joint action of benzoxazinone derivatives and phenolic acids. *J. Agric. Food Chem.* **2006**, *54*, 1049−1057.

(11) Macías, F. A.; Oliveros-Bastidas, A.; Castellano, D.; Marín, D.; El Mtili, N.; Molinillo, J. M. G. Influence of culture conditions on the exudation and assimilation of benzoxazinones. In *Proceedings of the 4th World Congress on Allelopathy*; Harper, J. D. I., An, M., Wu, H., Kent, J. H., Eds.; The Regional Institute Limited: Gosford, Australia, 2005; pp 395−399.

(12) Macías, F. A.; Oliveros-Bastidas, A.; Marín, D.; Castellano, D.; Molinillo, J. M. G. The allelopathic phenomenon, a dynamic process. In *Proceedings of the 4th World Congress on Allelopathy*; Harper, J. D. I., An, M., Wu, H., Kent, J. H., Eds.; The Regional Institute Limited: Gosford, Australia, 2005; pp 77−85.

(13) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/in.asp.

(14) Schulz, M.; Wieland, I. Variation in metabolism of BOA among species in various field communities—biochemical evidence for co-evolutionary processes in plant communities? *Chemoecology* **1999**, *9*, 133−141.

(15) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Molinillo, J. M. G. Optimization of benzoxazinones as natural herbicide models by lipophillicity enhancement. *J. Agric. Food Chem.* **2006**, *54*, 9357−9365.

(16) Macías, F. A.; De Siqueira, J. M.; Chinchilla, N.; Marín, D.; Varela, R. M. Molinillo, J. M. G. New herbicide models from benzoxazinones: aromatic ring functionalization effects. *J. Agric. Food Chem.* **2006**, *54*, 9843−9851.

(17) Marín, D. Development of natural herbicide models from benzoxazinones. Ph.D. Thesis, University of Cádiz, 2006.

(18) Perlak, F. J.; Oppenhuizen, M.; Gustafson, K.; Voth, R.; Sivasupramaniam, S.; Heering, D.; Carey, B.; Ihrig, R. A.; Roberts, J. K. Development and commercial use of Bollgard® cotton in the USA—early promises versus today's reality. *Plant J.* **2001**, *27*, 489−501.

(19) Coja, T.; Idinger, J.; Blümel, S. Influence of the soil composition on the effects of benzoxazinoid allelochemicals on two soil nontarget organisms. *J. Agric. Food Chem.* **2006**, *54*, 1093−1098.

(20) Coja, T.; Idinger, J.; Blümel, S. Effects of the benzoxazolinone BOA, selected degradation products and structure related pesticides on two soil organisms. *Ecotoxicology* **2006**, in press.

(21) Idinger, J.; Coja, T.; Blümel, S. Effects of the benzoxazinoid DIMBOA, selected degradation products and structure related pesticides on soil organisms. *Ecotoxicol. Environ. Saf.* **2006**, in press.

(22) Fritz, J. I.; Braun, R. Ecotoxicological effects of benzoxazinone allelochemicals and their metabolites on aquatic nontarget organisms. *J. Agric. Food Chem.* **2006**, *54*, 1105−1110.

(23) Lo Piparo, E.; Smiesko, M.; Mazzatorta, P.; Benfenati, E.; Idinger, J.; Blümel, S. Preliminary analysis of toxicity of benzoxazinones and their metabolites for *Folsomia candida*. *J. Agric. Food Chem.* **2006**, *54*, 1099−1104.

(24) Lo Piparo, E.; Fratev, F.; Lemke, F.; Mazzatorta, P.; Smiesko, M.; Fritz, J. I.; Benfenati, E. QSAR models for *Daphnia magna* toxicity prediction of benzoxazinone allelochemicals and their transformation products. *J. Agric. Food Chem.* **2006**, *54*, 1111−1115.

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Received for review August 17, 2006.

JF062377F