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Special Feature

Remembrances of Boll Weevil Research: Identification of the Boll Weevil Feeding Stimulant

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This article is dedicated to the late Dr. William L. Parrott, a good friend and co-worker

It's amazing how many discoveries are the results of serendipity. Such was the case with our identification of the feeding stimulant for the boll weevil at the USDA-ARS Boll Weevil Research Laboratory in the mid-eighties. We always knew there were chemical components of the cotton plant that elicited a feeding response. It was equally apparent that there were other, volatile, chemicals that functioned as attractants. An attractant is by nature a volatile material and is usually a component of the essential oil of the host plant in question (in the case of plant feeders). It causes a positive orientation to the feeding source. In the case of the boll weevil, this was easily demonstrated in the laboratory olfactometer, which played a major role in the identification of the male-produced pheromone for this species. In this two-choice assay, weevils consistently crawled a few centimeters into the side pocket containing a water suspension of ground cotton squares.

A feeding stimulant is defined as a chemical that causes a feeding response; in the case of the boll weevil, this was observed as a probing and penetration of the substrate. Our early feeding assays were conducted by wrapping 2-cm agar cylinders with filter paper rectangles containing the candidate material, and then counting the number of punctures after a prescribed length of time. I learned a lot about bioassays from Jack Keller, for whom I worked in the Toxicology Laboratory at the Boll Weevil Research Laboratory in the early sixties. Jack had reported the presence of an extractable principle in cotton squares that elicited an arrestant and feeding response by the boll weevil (Keller, J.C., Maxwell, F.G., and Jenkins, J.N. 1962. Cotton extracts as arrestants and feeding stimulants for the boll weevil. J. Econ. Entomol. 55: 800-801.)

Feeding stimulants, as a group, tend to be nonvolatile compounds. This is understandable since olfaction plays little or no role in their detection, and they are detected by tarsal contact. In early efforts at developing a bait, it was quickly apparent that boll weevils liked sugar. In fact, a surprising observation was made that boll weevils tend to like whatever tastes good to humans. When assaying candidate feeding repellents, the most effective materials taste bad to us also, often being bitter. We once published a paper (McKibben, G.H., Hedin, P.A., Davich, T.B., Daum, R.J., and Laseter, M.W. 1971. Addition of Food Acidulants to increase attractiveness to boll weevils of bait containing cottonseed oil. J. Econ. Entomol. 64: 583-585.) showing that food acidulants (for example, organic acids such as citric acid found in citrus fruits and malic acid found in apples) extensively used in food products to enhance flavor also enhanced the feeding response of the boll weevil. (This principle is one reason citric acid is used in soft drinks and why we like lemon and lime juice with so many dishes) But the fact that the boll weevil shares similar taste preferences with humans always seemed to me a strange and totally unexpected observation.

Since sugars are found universally in plants, we assumed that there must be very specific compounds in the cotton plant that cause the boll weevil to feed on it. We didn't set out to identify the feeding stimulant at first. Bill Parrott, who worked in the Host Plant Resistance Unit, was interested in identifying an oviposition stimulant for the boll weevil, so we worked together on the project, assaying extracts of cotton squares. For the oviposition assay, our procedure was similar to that used with feeding assays except that we had to dissect the agar pellets under a microscope and count eggs, a laborious procedure. We really never got anywhere with the oviposition stimulant work, but we did notice that some of our extracts elicited marked feeding responses, judging by the holes punched in the filter paper wrappers. It was at this point that I started off in another direction in an attempt to isolate and identify the active feeding stimulant compound or compounds.

Because some of our prepared fractions obviously contained a feeding stimulant, I applied an outdated method called paper chromatography for our first separations. I applied a thin line of the extract near the bottom of a sheet of chromatography paper and placed it into a chromatography jar containing an appropriate solvent. This causes the solvent to migrate up the paper, taking the chemicals along with it to varying distances. A separation occurs as some chemicals adhere more strongly to the paper than others. The result is a series of bands, not always visible without treatment for UV examination, representing the various compounds or, most often, mixtures of similar compounds. This method had been replaced by column, thin-layer plate, and gas chromatography. Normally, the fractions represented by bands on the paper were cut out with scissors and re-extracted for bioassay, but since our feeding assay involved boll weevils punching holes in filter paper, this method allowed a rapid and novel way to identify the active fractions without any cutting or extracting.

What we did was to take the paper out of the jar when the solvent front reached near the top, allowed the solvents to evaporate, and placed the paper directly into a shallow dish into which agar had been poured and allowed to congeal. Then boll weevils were placed into the dish and allowed to find the band where they wanted to feed. The first time we did it a certain band on the paper was riddled with holes. Thus a relic from the past gave us our first major progress on the project, and it was at this point that I contacted the Chemistry Unit at the Boll Weevil Lab.

At that time the Chemistry Unit was headed by Paul Hedin, who, along with A. C. Thompson and a post-doc from Switzerland named Giles Nicolier, comprised the professional staff. I found it much easier to interest chemists in a project if we have already made some progress, which in this case we had with the initial separations. An absolute necessity in these cooperative efforts is that the biologist has a dependable bioassay worked out.

A. C. and Giles agreed to try to identify the active feeding stimulant chemicals, which seemed to be concentrated in the anthers. Giles' specialty was column chromatography. I had never seen anyone break a chromatography separation into so many fractions. He taught me to do the column separations, and I would have at least two dozen test tubes, labeled A, B, C, … into which 5 ml fractions would be collected. Since Giles was busy on other projects as well, he depended on me to do many of the separations. I would then assay them and report the results back to him. Typically when one of the fractions was found to be active, he would design a column method to further fractionate that fraction, which was usually composed of many compounds. This work is tedious and time-consuming, frequently requiring several years to complete. You have to have patience, but for me it was also exciting and rewarding.

One day A. C. and Giles came down and told me it looked like the active compounds were lipids. Lipid chemistry, as far as identification was concerned, was a highly specialized field, with only a handful of "experts." One of the best, if not the very best, they told me, was Malcolm Thompson, a USDA-ARS chemist in Beltsville, Maryland. I contacted Malcolm and thus began what turned out to be a two-year, and sometimes frustrating, experience in identifying the elusive compounds. Sometimes my interest flagged, and he would try to get me interested again, and the reverse was also true.

I never met Malcolm in person, but in the many, many hours spent on the telephone with him over the next two years (we didn't have email back then) it was obvious that I was dealing with not only an outstanding chemist, but one who was a Christian gentleman as well. He told me about his upbringing in a poor neighborhood in Louisiana. Someone recognized his potential and arranged for him to attend high school, a privilege not generally available to black kids where he grew up. He told me this was a ministry of a local church, to which he would ever be grateful. His upbringing had instilled in him admirable qualities admired by all.

After the identifications had been made and confirmed and it was time to prepare a paper for publication, I wrote a rough draft and forwarded it to Malcolm, who revised and improved it to the extent that it contained more of his writing than mine. We planned to submit it to the *Journal of Chemical Ecology*, and I insisted that he be the senior author. He modestly declined, saying he didn't need any more papers, and it was published (McKibben, G.H., Thompson, M.J., Parrott, W.L., Thompson, A.C., and Lusby, W.R. 1985. Identification of feeding stimulants for boll weevils from cotton buds and anthers. J. Chem. Ecol. 11: 1229-1238) with me as the senior author, even though I still felt that he should have been listed first. A spin-off paper was published (Lusby, W.R., Oliver, J.E., McKibben, G.H., and Thompson, M.J. 1987. Free and esterified Sterols of cotton buds and anthers. Lipids. 22:80-83) with me a junior author, reporting on the lipid make-up of cotton buds and anthers. Typically, Malcolm was not the senior author on this one either. Bill Parrott later studied ester extracts of several Malvaceous plants and found a quantitative relationship between esters in hexane extracts and feeding response by boll weevils, regardless of plant species (Parrott, W.L., McKibben, G.H., Robbins, J.T., and Villavaso, E.J. 1989. Feeding response of the boll weevil (Coleoptera: Curculionidae) to ester extracts of host plants. *J. Econ. Entomol.* 82: 449-453).

The feeding stimulant turned out to be a mixture of esters of long-chain fatty acids. They were, fortunately for me, easy to synthesize in the laboratory, which I was able to do after Malcolm gave me the recipe. Soon after confirmation in the assays, it occurred to me that we might be able to formulate an all-synthetic bait. We had the synthetic pheromone grandlure, we now had a synthetic feeding stimulant, and I had had some experience formulating PVC (polyvinylchloride). So I formulated plastic pellets that were cured in such a way that they were friable and could actually be ingested by the boll weevil, much as they do a cotton square. I incorporated the yellow-green pigment used in the boll weevil trap. It was exciting to see weevils crawl over to a plastic pellet and begin to feed on it. A patent was applied for and obtained (McKibben, Gerald H., Dickens, Joseph C., and Smith, James W. 1994. Plastic bait composition for attracting and killing crop pests. U.S. Patent 5290556). There was nothing previously in the literature about any all-synthetic bait, and as patents go, it was a relatively easy process. The practical outcome of the work was the development of the boll weevil *Bait Stick*, which is another story.