The next task was to determine which specific oligosaccharide from the fungus cell wall caused plant cells to make phytoalexins. Because the number of different arrangements of oligosaccharides in the fungal cell wall material under investigation is so great, the subsequent project—the search for the active oligosaccharide—proved to be extremely challenging.

First, the oligosaccharides were separated according to the number of glucose units they had. It was determined that the smallest fungus oligosaccharide capable of stimulating the synthesis of phytoalexins was made up of seven glucose units. But the fungal cell wall matrix had more than 300 seven-glucose oligosaccharides, that is, the seven glucoses were arranged in more than 300 different patterns. The much more thorny question, then, and one which would require almost 10 years and great advances in analytical instrumentation to answer, was which one of these 300 different oligosaccharides activated phytoalexin production in the plant cell.

This task was particularly difficult because the oligosaccharides, though different structurally, are very similar chemically. Several of the oligosaccharides had a backbone chain made up of five glucose units with two single glucose branches attached. These oligosaccharides differed from one another only in where the branches were attached to the backbone.

Beginning in 1974 and continuing until 1983, researchers attempted to isolate and purify the oligosaccharide that stimulated the phytoalexin response. Finally, in 1983, 50 millionths of a gram (about one-tenth of a grain of salt) of the active oligosaccharide—the oligosaccharin—was successfully purified. Thanks to advances in analytical instrumentation, this small amount was enough to decipher the structure of the oligosaccharin molecule—an accomplishment that was impossible with 1974 technology, which demanded much larger samples.

The results established that a plant (in this case soybean) recognizes and responds to a specific oligosaccharide structure. The branch positions determine which oligosaccharide is capable of stimulating a response in plant cells. The presence of only a billionth of a gram of the active form is enough to cause the plant to synthesize phytoalexins which then inhibits the growth of the fungus from which the oligosaccharide originated. The plant cell is thus protected from further effects of the fungus.

Once it was recognized that oligosaccharides play a key role in eliciting defense responses, the possibility that they might stimulate the development of the various parts of plants was also addressed. Several different mixtures of oligosaccharides were added to slivers of tobacco-plant stems, called explants, growing in a tissue culture medium. One mixture was found to inhibit flowering and stimulate prolific budding for new leaves. In the presence of a different mixture, other explants that normally formed buds for new leaves formed flowers instead. Still another oligosaccharide mixture caused explants that would ordinarily have formed buds for new leaves to form prolific roots. It was found that the oligosaccharides were effective in impressively low amounts and that oligosaccharides with very specific structure were required to observe the regulatory activities. The oligosaccharin that induces flowers has been purified and structurally characterized. The small active molecule in this case consists of 10 sugars.

Thus, Basic Energy Sciences research revealed that oligosaccharides obtained from the plant cell wall can serve as a powerful family of chemical messengers. Although similar chemically, they exhibit broad structural diversity with different structural forms delivering different messages that turn on or express the genetic material that regulates plant function. The functions include growth, defense against disease, and cell differentiation in the course of development; i.e., whether to form roots, stems, leaves, or flowers.

While the mechanism by which oligosaccharins influence expression is not yet fully resolved, researchers are already exploring applications of important potential benefit. One day it may be possible to spray specific oligosaccharins on plants to tell them to flower; to form seeds, fruits or tubers; to become resistant to a disease or an insect; to drop their fruit; to grow faster; or to become bushier. Oligosaccharins may eventually have a profound impact on agricultural productivity and on the production of renewable resources for fuels and chemicals.