Perspective

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Pesticidal natural products – status and future potential

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Abstract

There is a long history of using natural products as the basis for creating new pesticides but there is still a relatively low percentage of naturally derived pesticides relative to the number of pharmaceuticals derived from natural sources. Biopesticides as defined and regulated by the US Environmental Protection Agency (EPA) have been around for 70 years, starting with *Bacillus thuringiensis*, but they are experiencing rapid growth as the products have got better and more science-based, and there are more restrictions on synthetic chemical pesticides. As such, biopesticides are still a small percentage (approximately US$3–4 billion) of the US$61.3 billion pesticide market. The growth of biopesticides is projected to outpace that of chemical pesticides, with compounded annual growth rates of between 10% and 20%. When integrated into crop production and pest management programs, biopesticides offer the potential for higher crop yields and quality than chemical-only programs. Added benefits include reduction or elimination of chemical residues, therefore easing export, enabling delay in the development of resistance by pests and pathogens to chemicals and shorter field re-entry, biodegradability and production using agricultural raw materials versus fossil fuels, and low risk to non-target organisms, including pollinators. Challenges to the adoption of biopesticides include lack of awareness and education in how to deploy their unique modes of action in integrated programs, testing products alone versus in integrated programs, and lingering perceptions of cost and efficacy.

Keywords: natural products; biopesticides; biocontrol; microbial control; integrated pest management

1 DEFINITIONS AND REGULATIONS

Biologicals comprise three general categories: (i) biopesticides ('biocontrol' is used outside of the USA), (ii) biostimulants and (iii) biofertilizers. Biopesticides, regulated by the Biopesticide Pollution Prevention Division (BPPD) of the US Environmental Protection Agency (EPA), are used for crop protection and plant growth regulation, and are further defined below. Biostimulants, regulated by states rather than the EPA, are used to increase plant health and reducing crop stress. There is no universal definition for biostimulants, but most refer to this term for products that increase crop growth and yields, and manage abiotic crop stress. The pending US Farm Bill defines a 'plant biostimulant' as a 'substance or microorganism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield.' The European Biostimulant Coalition (EBIC) recently announced a breakthrough in the agreed on regulatory framework for biostimulants (http://www.biostimulants.eu/2018/11/ebic-welcomes-compromise-reached-at-trilogue-meeting-on-fertilising-products-regulation-first-step-towards-eu-wide-market-creation-for-biostimulants/).

Some examples of biostimulants are seaweed extracts and some microorganisms. Since plant growth regulators (PGRs) are regulated in the BPPD, there is some confusion as to what truly defines a PGR versus a biostimulant. Some biostimulants contain plant hormones that regulate plant growth, causing confusion about what should be EPA regulated versus state regulated as a biostimulant.

Biofertilizers provide crops with crop nutrition such as N, P, K and micronutrients. They are comprised of humic acids and other natural substances, and some microorganisms or mixtures of these. Like biostimulants, biofertilizers are regulated state by state.

1.1 Biopesticides

The US EPA defines biopesticides as pesticides derived from natural materials. There are three branches of the BPPD for the three categories: biochemical pesticides, microbial pesticides and plant-incorporated protectants.

1.1.1 *Biochemical pesticides*

Biochemical pesticides contain naturally occurring substances that control pests. Substances that control diseases include potassium bicarbonate, phosphorous acids, plant extracts, pheromones for insect mating disruption and botanical oils. Not all natural biochemicals are regulated as biopesticides. The EPA requires the registrant to prove that the substance has a non-toxic mode of action to the pest or pathogen**.** A petition must be submitted to the Biochemical Classification Committee in the BPPD of the EPA. This requirement causes a great deal of confusion – how can you kill a pest without a toxic mode of action? Even if it is low risk to non-target organisms, the mode of action speaks to the effect on the target pest or pathogen. Examples of non-toxic modes of action include induced systemic resistance and systemic acquired resistance for control of plant pathogens (knotweed, seaweed

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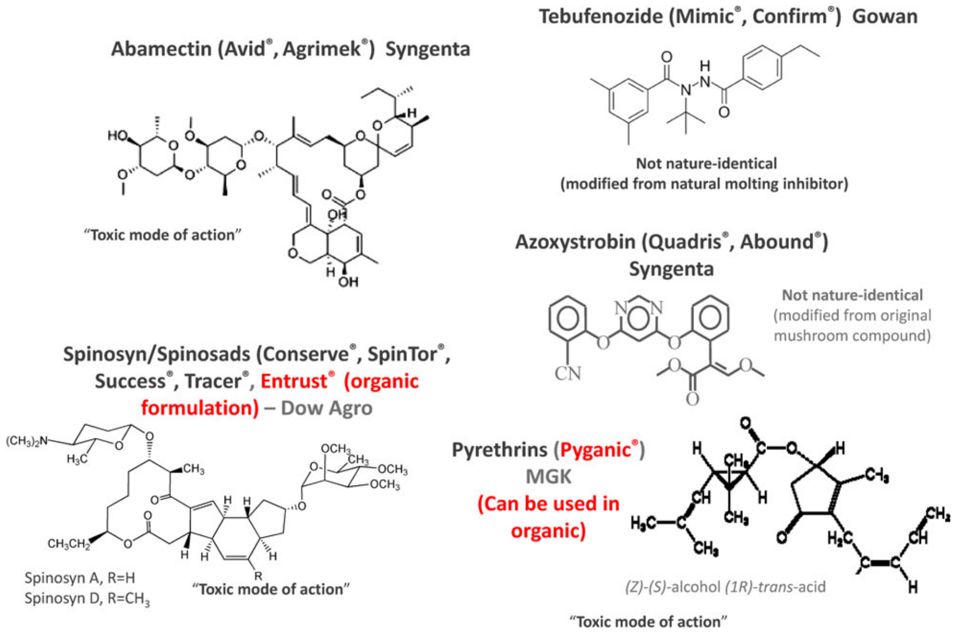


Figure 1. Pesticidal natural products and pesticides derived from natural products registered as chemical pesticides, not as biopesticides.

extracts), suffocation and desiccation (diatomaceous earth, oils), growth regulation (neem-based products) and mating disruption pheromones. Therefore, substances that are natural with a toxic mode of action are regulated as chemicals. Examples include the spinosyns (produced in fermentation), avermectin (produced in fermentation) and pyrethrins (extracted from plants), which all have toxic modes of actions because they work specifically on the insect's nervous system with some cross-over to mammalian systems. Also note that these three products are purified and concentrated from their natural state, increasing the toxicological risk. Figure 1 shows examples of natural products that are all regulated as chemical pesticides because they either have a toxic mode of action (to the pest) or are modified synthetically and therefore are no longer nature identical, eliminating them from being regulated as biopesticides.

1.1.2 *Microbial pesticides*

Microbial pesticides contain microorganisms (bacteria, insect viruses, fungi, actinomycetes, protozoa, etc.) that function as biocontrol agents, affecting the pest directly or indirectly through the compounds they produce. The best-known and largest microbial biopesticide is of course *Bacillus thuringiensis* (Bt), having been commercialized for more than 70 years. The microorganisms regulated under the EPA's microbial branch can be dead or alive. The inclusion of dead microorganisms allows for innovation around Gram-negative bacteria without hardy and stable resting spores found in groups such as *Bacillus*. Examples include Marrone Bio Innovations' bioinsecticides based on a new species of bacteria, *Chromobacterium subtsugae* and *Burkholderia rinojensis*, and Valent Bioscience's nematicide from *Myrothecium verrucaria*.

1.1.3 *Plant-incorporated protectants*

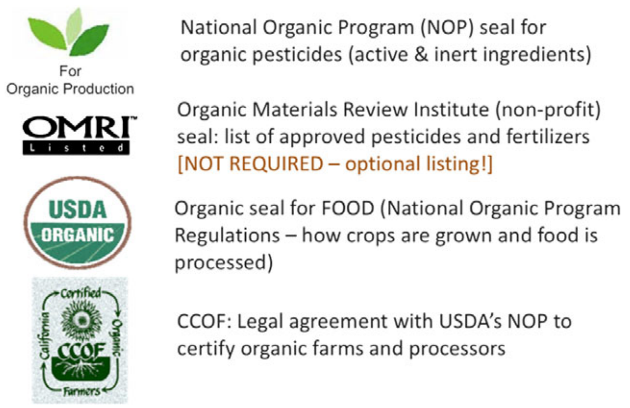
Plant-incorporated protectants (PIPs) are pesticidal substances produced by plants that contain genetic material added to the plant, often through genetic engineering. The EPA regulates the genetic material and the protein it encodes, but not the plant itself. PIPs include crops engineered to contain a gene that codes for the production of insect-killing proteins from Bt and virus-resistant plants that produce a virus-coat protein, which covers virus particles after infection and prevents their replication. PIPs will not be discussed in this paper as they are generally not considered biopesticides although they are regulated in the BPPD.

The EPA tiers the data requirements for both chemicals and biopesticides (microbials and biochemicals). Companies wishing to submit a dossier to the EPA's BPPD must submit Tier I toxicology and ecotoxicology (called a 'six pack'):

- rat acute studies - oral, inhalation, intravenous, dermal
- rabbit eye
- guinea pig skin sensitization
- product chemistry, five-batch analysis
- microbiology/QC (Quality Control): no human pathogens
- ecological effects (non-target birds, fish, *Daphnia*, honeybees, lacewings, ladybeetles, parasitic wasps).

If there are no direct toxic effects in this first tier, then second and third tier studies are usually not required (except for honeybees). This is advantageous to small biopesticide companies as the financial capital required to prepare a registration dossier is affordable (typically less than US$3 million). Under the Pesticide Registration and Improvement Act (PRIA) passed unanimously by Congress in 2001 and since reauthorized several times, lower submission fees are required for small businesses and biopesticides than for large companies and synthetic chemicals, which is cost prohibitive for small companies.

Harmonization with Canada has had limited success because Canada asks for more toxicology data than required by the EPA. Submission of a product concurrently to both the EPA and the Pest Management Regulatory Authority (PMRA) of Canada for joint review does not reduce the time for approval, and often increases it. EU biopesticide regulations are more cumbersome than those in the USA; the EU would like to accelerate more biological tools to the market. In 2014 the EU passed the Sustainable Use Directive, and due to the subsequent restriction and elimination of so



National Organic Program (NOP) seal for organic pesticides (active & inert ingredients)

Organic Materials Review Institute (non-profit) seal: list of approved pesticides and fertilizers [NOT REQUIRED – optional listing!]

Organic seal for FOOD (National Organic Program Regulations – how crops are grown and food is processed)

CCOF: Legal agreement with USDA's NOP to certify organic farms and processors

Figure 2. Description of logos for organic inputs, organic food and farming.

many chemical active ingredients, biopesticides are assessed on a case-by-case basis that costs several millions of dollars more than a US registration and takes several more years for approval.[1] Note that all countries except the USA require efficacy data for registration. California's Department of Pesticide Regulation does its own review, which could take up to 18 months after EPA approval. California also requires efficacy data.

1.2 Biopesticide use in organic production

Nearly all biopesticides are approved for organic production under the National Organic Program (NOP) and most products approved for organic production are biopesticides. When a registrant submits a biopesticide to the EPA, the BPPD, under an agreement with the NOP, reviews the active ingredient, formulation and manufacturing process for compliance with the NOP. On the biopesticide container, the EPA allows a triple leaf logo (see Fig. 2), indicating the product is acceptable for use in organic production. The Organic Materials Review Institute (OMRI) also reviews and lists substances and products (including biopesticides, biostimulants and biofertilizers) for organic production. While not required for biopesticides if the product is listed under the NOP after EPA approval, most growers recognize the OMRI logo (Fig. 2) and therefore companies typically get both listings.

There are some exceptions where biopesticides are not allowed in organic production because the active ingredient is made synthetically, although it is nature-identical. For example, Requiem® insecticide (Bayer Crop Science) uses synthetic terpenes rather than terpenes extracted from the original plant, *Chenopodium ambrosioidies*, and therefore it is not listed for organic production and neither is Marrone Bio Innovations' herbicide (EPA approved but not yet commercialized) from sarmentine, originally discovered from the plant *Piper longum,* but made synthetically (to reduce cost of goods). Another example is phosphorous acids used for control of plant pathogens. There are exceptions. For example, pheromones for mating disruption are made synthetically but are allowed in organic production because at this time there is no other way to manufacture them economically and they are very low risk.

Another reason for biopesticides to lack acceptance for organic listing is due to the inerts in the formulation. For example, potassium bicarbonate products like EcoMate®, Armicarb O®, Kaligreen® and MilStop® are approved, whereas Armicarb® (no longer manufactured) was not because it contained inert ingredients not allowed under the NOP.

There are important pesticides used in organic fungicides that are not biopesticides, including copper and sulfur. Their use in organic production is controversial because of the environmental buildup of copper, air, and respiratory and dermal effects of sulfur. They are classified as 'restricted materials' under the organic rules and they should only be used as last resort. Note that these two fungicides are also the most widely used by conventional farmers.

Some biopesticides are defined as minimum-risk pesticides through the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) Section 25(b) rule because their active and inert ingredients are low risk and generally recognized as safe (GRAS). Consequently, these are exempted from the EPA's FIFRA regulation requirements and can be used on any labeled crop and for non-crop use since they do not need to be registered as a pesticide. 'Exempt from EPA registration' is stated on the label of these products. These 25(b) substances include plant essential oils such as peppermint, wintergreen and clove oils. For use in organic farming, these 25(b) substances need OMRI listing.

2 THE ESTIMATED MARKET FOR BIOPESTICIDES AND BIOSTIMULANTS

The agriculture biologicals consulting and market research company Dunham Trimmer estimates the market for biopesticides between US$3 and uS$4 billion or about 5–6% of the total global pesticide market.[2] They project compounded annual growth (CAGR) at 17% with the fastest growth in Latin America, North America and Europe comprising 67% of global biopesticide sales in 2020. Microbials are projected at 58% of the total market and bioinsecticides are the largest category dominated by *Bacillus thuringiensis*. Bionematicides are the fastest growing category sparked by market need due to loss of toxic chemical nematicides. Eighty percent of the use of biopesticides is on fruits and vegetables (17.6% share of total pesticides). Bioherbicides have not yet broken out and remain a very small portion of the biopesticide sales. If you remove herbicides from the tally, biopesticides comprise 8.3% of the pesticide market compared to 5.2% when herbicides are included.

Dunham and Trimmer[2] estimate the biostimulant market at about US$2 billion with growth at about 12–15%. Like biopesticides, Latin America is the fastest growing region, with microbials and seed treatments growing most rapidly. The EU is the largest market at 35%, followed by North America (23%), Asia-Pacific (22%) and Latin America (18%). Seaweed extracts dominate at 37% of total biostimulants with Arysta (now part of UPL) and Valagro leading this sector. Large agrichemical companies and startups alike (funded by venture capital and private equity) are investing in biostimulants more than biopesticides because of the lower regulatory barriers to bring new products to market. As such, the market is very crowded and there is confusion by growers as to the quality of some products that are uncharacterized microbial mixtures. Like biopesticides, more science is being brought to this natural product category to distinguish the 'bathtub brews' from products with toxicology data and characterization of the key microorganisms and their associated natural compounds, and mode of action.

What is causing the faster global growth rate and increased adoption of biopesticides? They offer several key benefits:

1. Better yields and quality in integrated programs. While biopesticide developers have seen many trials and farmer demonstrations showing that their products and other biopesticides can perform as well as chemical pesticides on their own, particularly when measuring marketable yields, biopesticides are best used when incorporated into programs. Farmers rarely use anything stand-alone and typically mix and rotate a variety of pest management tools. They alternate products from spray to spray and often mix more than one product together in the spray tank. Farmers do this to get better results and to delay or stop the evolution of pest resistance (see 3 below). Because of the unique way that biologicals work, known as their modes of action, we often see that 1 + 1 = 3 instead of 2, meaning that combinations of chemicals and biologicals result in higher yields and better quality compared to chemical-only programs. For example, Regalia® (an extract of giant knotweed), commercialized by Marrone Bio Innovations as a biofungicide, has shown an increase in yields and quality on several crops.[3] For example, on corn, Regalia consistently yielded 775 kg per hectare more corn and 332–498 more kilos per hectare in soybeans when combined with the leading chemical fungicide.

For controlling insect pests such as the navel orangeworm, which is becoming resistant to some of the chemical insecticides used on almonds, use of mating disruption pheromones reduced insect damage.[4] In 2018, Marrone Bio Innovations combined each of its two microbial insecticides along with two of the leading chemical insecticides (each in a separate tank mixture) to increase the control from approximately 50% to above 90%, creating an estimated 20-fold return on investment for growers (Marrone Bio Innovations, unpublished data).

Recently, biologicals have seen a breakthrough technology in seed coatings (or seed treatments) to protect crops at planting time from destructive insects, nematodes (roundworms that feed on the roots of plants) and diseases. Microbial seed coatings (containing microorganisms such as *Pasteuria*, *Bacillus firmus*, *Bacillus subtilis* and Marrone Bio's *Burkholderia rinojensis*) stacked with chemical pesticides on the seed are now widely used on corn, soybean and cotton, and marketed by large agrichemical companies including Syngenta, Bayer, BASF and Albaugh.

Adding the biological has shown to increase yields above yields of the chemical-only seed treatments. Another two of Marrone Bio Innovations' bacteria (a new strain of *Bacillus amyloliquefaciens* and a new species of bacteria, *Chromobacterium subtsugae*) stacked with a beneficial, yield-enhancing mycorrhizae fungus from Israel (Groundwork BioAg) created an all-biological seed treatment that performed as well as or better than the all-chemical or the chemical-bio commercial standards in increasing yields of corn and soybeans.

2. Better science = better performance. Biopesticides have become better over time in performance and cost. Investment in science to find new strains and species of microorganisms with higher efficacy, application of genomics tools to understand the microorganisms and microbial physiology, more stable formulations, and entry by large agrichemical manufacturers has brought more legitimacy to the biopesticide category. As discussed earlier, growers are increasingly learning how to use biopesticides in integrated programs and seeing the results of enhancement of chemical pesticides in higher yields and quality than chemical-only programs.

3. Resistance management. Most of today's chemical pesticides have a single site of action, attacking one vulnerable metabolic pathway or process of the pest. Therefore, after repeated use of a chemical pesticide, pests can quickly evolve resistance to that product. When resistance occurs, pesticides do not perform as expected. Biopesticides typically have unique, complex and, usually, multiple modes of action, which means that pests and plant disease-causing pathogens are less likely to evolve resistance to them. Therefore, in more than 50 years of commercial use, incidences of resistance are rare. Examples include resistance by the diamondback moth after repeated frequent uses of sprayable *Bacillus thuringiensis*[5] and repeated frequent uses of codling moth granulosis virus in organic apple production in Europe.[6,7]

4. Managing residues. Pesticide residues (maximum residue levels, MRLs) are regulated by individual countries and via global rules (the Codex Alimentarius, or Codex), but buyers, including retail supermarkets and branded food companies, have imposed their own, often stricter, limits on chemical residues that regularly dictate zero measurable pesticide residues. Biopesticides, due to their generally low risk to consumers, are exempt from residue tolerances (the amount of chemical allowed on the crop at time of harvest) and, as such, can be used right up to harvest. When there is a pest or plant disease that shows up near harvest, a chemical may not be an option if the residue persists or is not allowed by buyers. Using a biopesticide for those last sprays provides the reassurance of crop protection and the ability to export without rejection by a buyer.

5. Safety, biodegradability and reduced carbon footprint. Biopesticides generally affect only the target pests or plant pathogens and pose little to no risk to birds, fish, beneficial insects, pollinators, mammals and other non-target organisms. They also pose minimal risk to workers and, as readily biodegradable products, do not pollute air and water. Most biopesticides can be applied with the lowest level of personal protection equipment (PPE), such as gloves and masks, and typically do not require special permitting and large buffer zones (prohibited use areas) around homes, schools, public spaces and water bodies. Many biopesticides, particularly fermented microbials and plant extracts, are manufactured using agricultural raw materials and manufacturing waste that could be used as fertilizer. Marrone Bio Innovations undertook an analysis of the carbon footprint of its three primary biopesticides and determined that their carbon footprint was substantially lower than comparative chemical pesticides. Scoring the best was Regalia, an extract of giant knotweed, because the knotweed, an invasive species, is largely harvested from wild populations.

6. Labor flexibility. Biopesticides have short worker re-entry times, typically 4 h, as opposed to many chemical pesticides that have re-entry intervals of days to weeks. In today's tight farm labor environments, farmers can increase worker and grower productivity and reduce labor costs by allowing faster re-entry times when using biopesticides. This allows spraying in the morning and doing other tasks, such as harvesting or pruning, the same day.

7. Most biopesticides can be used in organic production. This topic was discussed above, but it is important to note that biopesticides are typically pigeon-holed as 'organic-only' products, despite the fact that biopesticide and agrichemical companies sell most of their biopesticide products to conventional growers. Today, consumer demand for organic food exceeds supply and organic food continues to be the highest growth food segment in the USA and Europe (Table 1). There is a shortage of organic plantings, especially for grain, to meet demand. Organic is still a small percentage of the total farm acreage, and growers typically can make more money per hectare with organic commodities. Food companies and retailers are now initiating programs to support the transition of more farm area to organic production.[8] However, the biopesticide industry sells to conventional growers that are using all the aforementioned benefits of biopesticides to optimize their operations. Biopesticides, when used as part of an integrated pest

Table 1. Organic food sales growth from 2008 to 2017

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Organic food (US$ billions)	20 393	21 266	22 961	25 148	27 965	31 378	35 099	39 006	42 507	45 209
Organic (year on year growth %)	17.50%	4.30%	8.00%	9.50%	11.20%	12.20%	11.90%	11.10%	9.00%	6.40%
Total food (US$ billions)	659 012	669 556	677 354	713 985	740 450	760 486	787 575	807 998	812 907	822 160
Total food (year on year growth %)	4.90%	1.60%	1.20%	5.40%	3.70%	2.70%	3.60%	2.60%	0.60%	1.10%
Organic (% of total)	3.10%	3.20%	3.40%	3.50%	3.80%	4.10%	4.50%	4.80%	5.20%	5.50%

Data from the Organic Trade Association (2018), https://www.ota.com/resources/market-analysis.

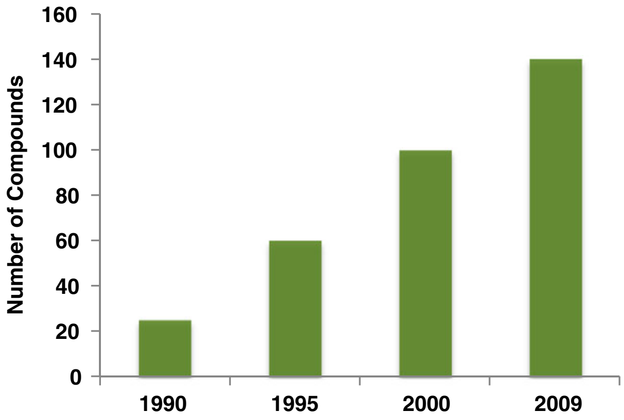


Figure 3. The increasing number of compounds screened to find one new chemical pesticide.

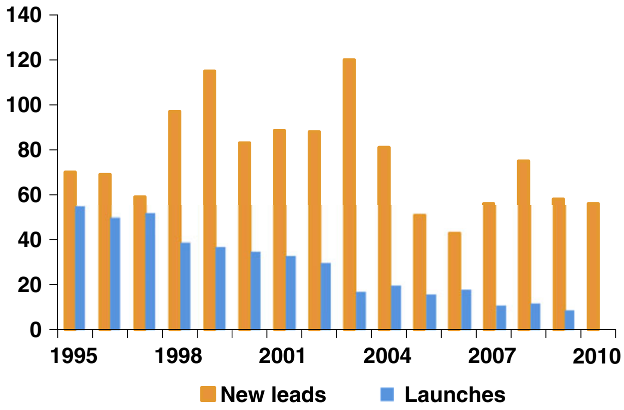


Figure 4. Number of new chemical leads versus number of synthetic pesticide product launches.

management strategy, provide all growers with maximum flexibility in meeting shifting consumer demands.

In contrast, the US$61 billion chemical pesticide market (US$53.7 billion crop protection and US$7.3 non-crop agrochemicals) is growing by single digits.[9] Herbicides constitute 40% of the total pesticide market value, insecticides 30% and fungicides 25%. The slow growth is due to chemical bans (such as the ban of neonicitinoids in Europe and Canada) and other regulatory restrictions around the world and the high cost and long period of time needed to develop a new chemical pesticide (nearly US$300 million and 12 years). Additional factors include historically low farm income in the USA and Brazil due to low row crop commodity prices and uncertainty of trade policies and the poor state of the Brazilian economy. The advent of corn and cotton crops engineered to contain Bt insect-killing proteins has also reduced chemical insecticide sprays.

The discovery of synthetic pesticides has become increasingly difficult and costly. It is estimated that companies must screen at least 140 000 chemicals to find one new, commercially acceptable, synthetic pesticide (Fig. 3).[10] The discovery of new chemical leads has decreased since 2005 and it is increasingly more difficult to convert a new lead into a new product launch, as indicated by the trending decline in new product launches from 2002 to 2010 (Fig. 4).[11] Since it now requires more than US$280 million to develop one new synthetic pesticide (Fig. 5) and takes nearly 12 years, fewer new chemical active ingredients are being launched.[10] In contrast, the cost to develop a biopesticide is in the order of US$3–7 million and it takes approximately 4 years or less to get to market in the USA.[12]

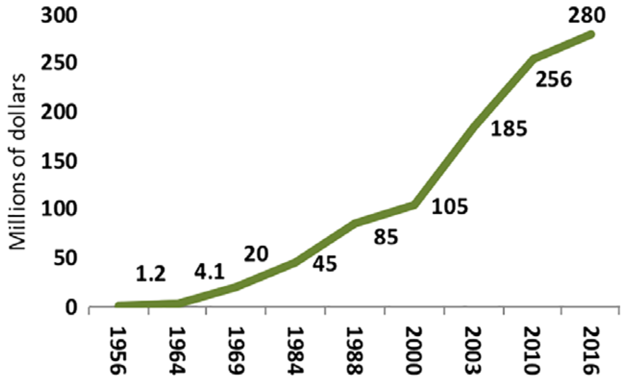


Figure 5. Cost and time to discover and develop a new synthetic chemical pesticide.

3 LARGE COMPANIES MOVE INTO BIOLOGICALS

Large agrichemical companies have become involved in biopesticides through in-licensing of technology and products, joint ventures and acquisitions. Table 2 lists the acquisitions or joint ventures of companies with a biologicals focus since 2009. These large companies have paid significant amounts to acquire companies with no or moderate revenues (e.g. Pasteuria, Devgen, Divergence, AgraQuest). In addition, several companies have started microbial biopesticide discovery programs, such as Monsanto and Novozymes in a joint venture called the BioAg Alliance (https://monsanto.com/news-releases/the-bioag-alliance-targets-250-500-million-acres-by-2025/) and FMC and Chr. Hansen (https://www.chr-hansen.com/en/media/2018/6/chr-hansen-and-fmc-

Table 2. Acquisitions and joint ventures of biological companies by larger companies and agrichemical companies since 2009

Company	Year acquired/Joint Venture	Price (US$ millions)	Acquirer or partner	Technology
EcoFlora	2019	Not disclosed	Gowan	Plant extracts for crop protection
Tyratech	2018	Not disclosed	American Vanguard	Essential oils for crop protection
Ginkgo Bioworks	2018	100	Bayer Crop Sciences	Synthetic biology to create microbes that can enhance nutrient uptake
Rizobacter	2016 (50.01%)	Not disclosed	BioCeres	Microbial inoculants for soybean and others
Novozymes	2014	300 into Novozymes	Monsanto	Biologicals joint venture with Bio-Ag Alliance
Chr. Hansen	2013	Not disclosed	FMC	Microbial screening joint venture
Novozymes	2013	Not disclosed	TJ Technologies	*Bacillus*-based plant health products
Center for Agricultural and Environmental Biosolutions	2013	Not disclosed	FMC	Microbial endophyte discovery
Prophyta	2013	35	Bayer	Fungi-based biopesticides
Devgen	2012	523	Syngenta	RNAi, rice germplasm
AgraQuest	2012	425 + 75 earnout	Bayer	Biofungicides, bioinsecticides
Pasteuria	2012	123	Syngenta	Bionematicide
Becker Underwood	2012	1000	BASF	Seed treatments, biopesticides
Divergence	2011	Not disclosed	Monsanto	RNAi, chemical nematicide
EMD	2011	275	Novozymes	Microbial inoculants
AgroGreen	2009	Not disclosed	Bayer	*Bacillus firmus* bionematicide

In addition, large companies have signed deals with smaller ones to gain access to technologies that they will distribute.

corporation-extend-collaboration-on-natural-crop-protection). Most recently, Bayer invested US$100 million to create a joint venture (Joyn) with Ginkgo Bioworks, a synthetic biology company (https://media.bayer.com/baynews/baynews.nsf/id/Bayer-Ginkgo-Bioworks-unveil-joint-venture-Joyn-Bio-establish-operations-Boston-West-Sacramento). In announcing the joint venture, Bayer expressed excitement about the opportunity to use synthetic biology to manipulate microorganisms in ways that are better than they are in nature. Pivot Bio recently raised US$70 million in new capital to advance its synthetically modified bacteria to produce nitrogen for monocots (https://www.prnewswire.com/news-releases/pivot-bio-closes-70-million-series-b-financing-to-deliver-first-and-only-clean-alternative-to-synthetic-nitrogen-fertilizer-for-us-corn-farmers-300722412.html). While synthetic biology is a powerful tool to manipulate and improve microorganisms, molecular and genomics tools can assist in improving manufacturing processes, yields and metabolite production of naturally occurring microorganisms as well. Marrone Bio Innovations has successfully increased certain metabolites produced by its insecticidal and nematicidal microorganisms more than 100-fold using traditional fermentation optimization enabled with knowledge of the full genome sequence of the microbe.

4 INVESTMENT IN BIOLOGICALS

Table 3 shows the robust activity in 2017–2018 of investment into companies in the biologicals industry.

Currently, living microbes are of great interest, with most investment going into startups with consortia of live, plant-colonizing microbes, soil health and synthetic biology of microorganisms. Most new companies do not have natural product chemistry groups and they therefore ignore the chemistry behind their products (except to use knowledge of gene sequences to understand what the microbe might be making). This is because it is often technically difficult to identify the complicated mixtures of compounds causing the pesticidal effect, and it is also expensive and may cause regulatory problems. Regulators typically require less toxicology data for products based on living microbes than for plant extract and dead microbial products. We of course know that microbes produce compounds, but regulators are much stricter with dead microbials and plant extracts, even when the risk of the compounds is very low and well known, such as extracts from food plants. The answer is not to make it harder to regulate living microbes, but to more sensibly balance the risks of highly biodegradable natural chemistry from microbes and plants. Unfortunately, more expensive requirements reduce innovation, as most investors and companies will focus on avenues of least regulatory time and cost. This is already being played out by the number of new startups focusing on biostimulants with consortia of microbes that claim an increase in plant health and yield versus natural product-based ones focused on crop protection.

5 NATURAL PRODUCT DISCOVERY AND DEVELOPMENT PROCESS

There are many ways to find new pesticidal natural products from microorganisms and plants. Some companies use genomic tools such as 16S RNA to screen and discover microorganisms of a particular taxonomy up front. Other companies, such as Marrone Bio Innovations, screen first for bioactivity against a target, and then identify and characterize the microorganisms. The following are

Table 3. Financing of agriculture biological companies in 2017 and 2018

Company	Investors	Size of round (US$million)	Year	What they do
AgBiome	The University of Texas Investment Management Company, Fidelity Management and Research Company, Polaris Partners, ARCH Venture Partners, Innotech Advisers, Pontifax Global Food and Agriculture Technology Fund and Monsanto Growth Ventures	65	2017	Microbials for pest management
Bioconsortia	Otter Capital and Khosla Ventures	10	2018	Consortia of microbes as biostimulants
Boost Biomes	Nimble Ventures, Viking Global Investors, Tencent	2.05	2018	Novel microbial biopesticide discovery platform
Concentric Ag (formerly Inocucor)	Cycle Capital Management, Desjardins Innovatech TPG ART and Pontifax AgTech	15.9	2018	Consortia of microbes as biostimulants
Indigo BioAg	Baillie Gifford, Investment Corporation of Dubai, the Alaska Permanent Fund and Flagship Pioneering	250	2018	Value-enhanced grain, seed and cotton with microbial inoculants
Marrone Bio Innovations	Ospraie, Waddell & Reed, Ardsley, Exponential & Public	40	2018	Biologicals for pest management and plant health
New Leaf Symbiotics	S2G Ventures, Monsanto Growth Ventures, Otter Capital, The Yard Ventures, Lewis & Clark Ventures, Rockport Capital, Pangaea Ventures, Open Prairie Ventures	30	2017	Pink-pigmented bacteria to enhance crop growth
Pivot Bio	Breakthrough Energy Ventures, Temasek	70	2018	Modified microbes for nitrogen fixation in corn
Semios	Sustainable Development Technology Canada	9.9	2018	Digital ag tools and pheromone biopesticides
Sound (formerly Asilomar Bio)	Syngenta Ventures Cavallo Ventures, Fall Line Capital and Cultivian Sandbox	12	2017	Strigalactone-related biostimulants
Terramera	Sustainable Development Technology Canada	2.5	2018	Formulation technology to improve biopesticides

typical steps in a classical pesticidal microbial natural product discovery process (also illustrated in Fig. 6) that has yielded several new species, novel compounds and new uses of known compounds, resulting in the development and commercialization of several biopesticide products.

5.1 Primary screening

5.1.1 Collection and isolation

Habitats and niches with high biodiversity are targeted to collect soil, compost, insects, flowers or other biological matter to isolate microorganisms on various agar media. For example, organic farms and rainforests are rich places to collect. When collecting around the world, companies often form collaborations in countries to comply with the Convention on Biological Diversity. The type of sample (flowers versus soil), storage conditions and time from collection until isolation, treatment of the sample in the laboratory, isolation methods and choice of media all have an effect on the types of microorganisms, their novelty and associated natural product compounds discovered. We typically rotate four to six media every few weeks to reduce the possibility of rediscovery of the same things over and again.

5.1.2 Fermentation

To test against various targets, we developed an automated miniaturized fermentation system for fermenting the test microbes in 50-mL centrifuge tubes. The microbes are fermented for 3 to 5 days (one microbe per media type). We then centrifuge the tubes and test the supernatant to skew our discovery for metabolite producers. As in the initial plate isolation, the fermentation media are rotated to increase the novelty of the compounds produced by the microbe in its cells or excreted into the media. We did not see an advantage of testing in more than two media recipes each week because a certain throughput is needed, therefore we chose to balance numbers tested versus number of media. We focused on fermentation media and processes designed to replicate those that would be required for large-scale fermentation and commercial production, avoiding the time and expense of an unsuccessful scale-up.

5.1.3 Bioassays

We developed several miniaturized bioassays for testing against insects, fungal and bacterial plant pathogens, weeds, plant parasitic nematodes and algae. The targets were chosen for their market size, need and potential, and ease of testing in the laboratory. Ideally, the economic pest itself is the screened organism, such as our choice of *Spodoptera exigua* (beet armyworm) and *Lygus hesperus* (tarnished plant bug), two particularly difficult insects to control. We tested against both fungal and bacterial plant pathogens and toxic algae, with few, if any, non-toxic solutions available in the market. We compare results to synthetic chemical pesticide standards.

Most bioassays are conducted in 24-, 48- or 96-well plates. Over time, we have changed bioassay methods if needed to increase the hit rate. In the case of the discovery of *Bacillus amyloliquefaciens*

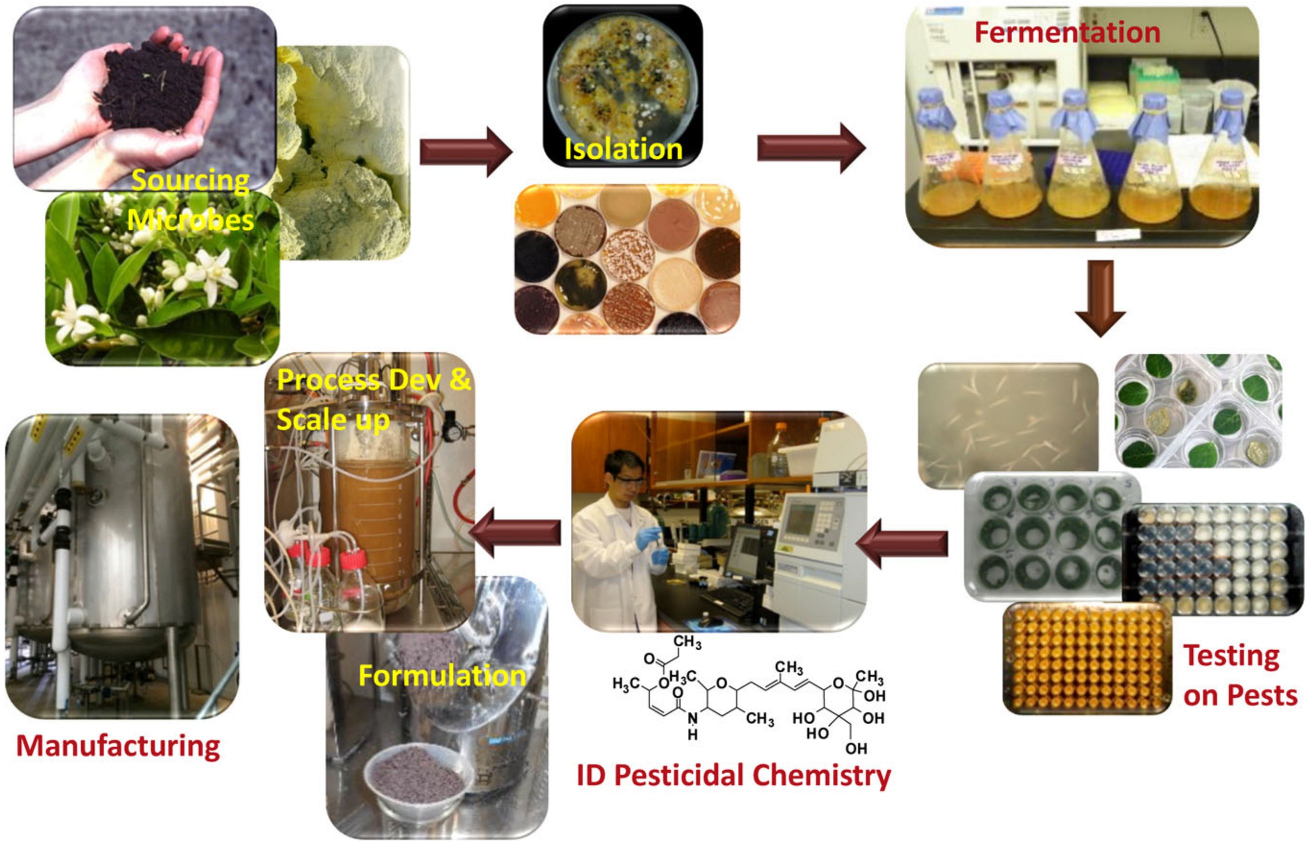


Figure 6. The process of discovery and development of microbial natural products (biopesticides).

F727, we determined that there was a market need for new products for control of Oomycetes (downy mildews and late blight, for example), but also broad-spectrum control against difficult pathogens such as *Botrytis, Venturia* and *Sclerotinia*. We screened nearly the entire collection (more than 16 000 microorganisms) against *Botrytis* and *Venturia* in 24-well plates with agar media. The best candidates were then tested against *Peronospora* (can only do this *in vivo*) and *Phytophthora*. The best strain, *Bacillus amyloliquefaciens* F727, had the strongest and broadest activity against these pathogens and was commercialized by Marrone Bio Innovations as Stargus® and Amplitude™. The activity against *Botrytis* and *Venturia* is due to novel lipopeptides, while the Oomycete activity is due to other peptides. Figure 7 shows the unique chemistry profile compared to other *Bacillus* biofungicides. Because there are so many strains of *Bacillus amyloliquefaciens* and other species of *Bacillus* deployed as biofungicides, some of which are patented, the chemistry profiles of any new products need to be compared to insure novelty of the intellectual property.

Although miniaturized assays on the pests themselves has proven the best way to discover commercial bioactivity, sometimes we developed assays based on a specific mode of action. For example, we developed and patented an assay against glutamine synthetase to find systemic herbicidal compounds using the discovery of glufosinate, originally from *Streptomyces* spp., as a model system for herbicide discovery.[13] With this method we discovered the herbicidal activity of the new species *Burkholderia rinojensis* A396 (submitted to the EPA) in parallel with its insecticidal activity on *Spodoptera exigua* in a 96-well plate diet-based bioassay (commercialized as Venerate®). The chemistry of this bacterium is discussed below.

After we find pesticidal activity, we test in a plant growth assay looking for microorganisms that have 'dual use', i.e. both pesticidal and plant health effects, to increase the value for our target farmer customer. When a microorganism shows a high level of pesticidal activity, we conduct further tests to determine the spectrum of activity, mode of action, stability and activity on plants.

Table 4 shows the hit rate of our tested microorganisms by pest target category (Marrone Bio Innovations, unpublished). It is very difficult to find bioactivity against insects compared to finding fungicidal activity or biostimulant (plant growth) activity. As a result, there are many new biofungicides and biostimulants being launched on the market but very few new insecticides. Nematicides are somewhere in between and the number of new microbial nematicides on the market reflects this intermediate difficulty in finding a commercial level of bioactivity. Therefore, one way to increase the number of insecticides is to increase the number of microbes tested against insects to find new biopesticide products.

5.1.4 *Natural product chemistry*

We use classical bioassay-guided fractionation to characterize the metabolites produced in the cells or excreted into the medium. The usual tools are deployed, including high-performance liquid chromatography (HPLC) with diode array detection technology, liquid chromatography-mass spectroscopy (LCMS), and gas chromatography-mass spectroscopy (GC-MS). We compare the natural product compounds produced by each of the selected microorganisms with known compounds in purchased and our own natural compound databases. This allows us to eliminate

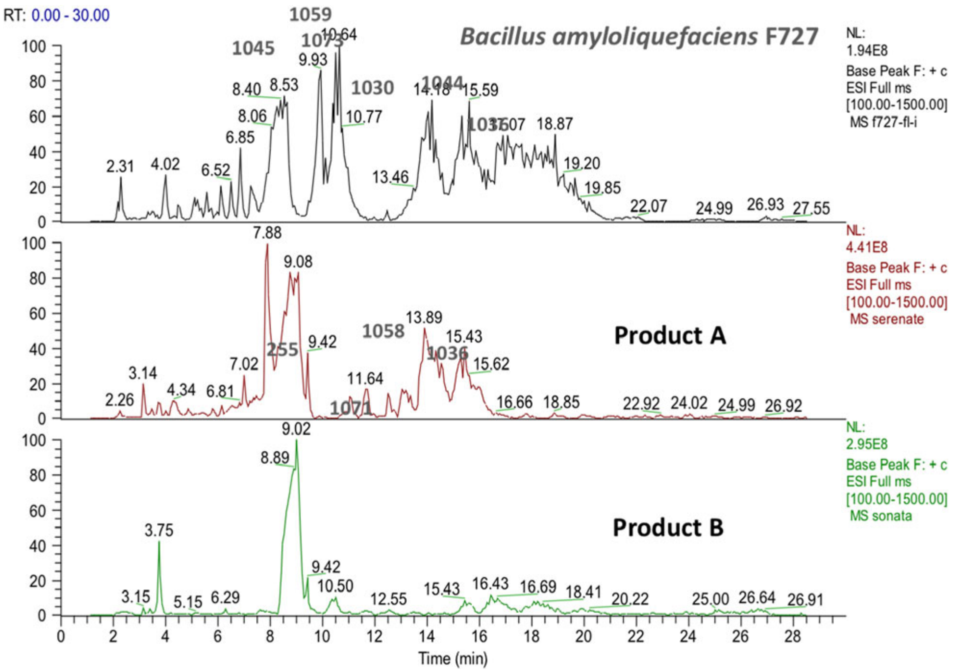


Figure 7. Comparing the chemistry profile of *Bacillus amyloliquefaciens* strain F727 with other *Bacillus* products.

Table 4. Data showing the hit rate (bioactivity) of various screened microbes by target category

Hit type	Total #	Hit rate (%)	One hit per....	Number screened
Herbicide, leaf disc	305	1.95	51	15 670
Herbicide, grass seedling	151	1.19	84	12 695
Herbicide, plant test grass	63	2.32	43	2721
Herbicide, plant test broadleaf	19	0.70	144	2729
Insecticide, beet armyworm	16	0.10	1002	16 037
Insecticide, *Lygus*	8	0.06	1568	12 547
Insecticide, corn rootworm	2	0.72	138	276
Fungicide, *Phytophthora*	954	5.74	17	16 620
Fungicide, *Monilinia* or *Botrytis*	940	5.65	16	16 620
Nematicide	206	2.0	50	9695
Algaecide	83	0.67	150	12 419
Bactericide	74	1.38	73	5371
Plant health (corn)	108	7.72	13	1399

those microorganisms that produce known toxins and to select those that we believe are novel and safe. From the selected microorganisms, we identify and characterize the natural product compounds responsible for their pesticidal activity. None of this is very straightforward as typically the metabolites are multiple classes of chemistry with multiple analogs per chemical class, and there are many synergistic activities among the compounds. One compound could be weak in activity itself but without it the total activity goes down. It may take months to years to fully characterize the pesticidal activity of a whole cell broth to understand the synergies among compounds. Fortunately, today's genomic tools and information about the gene sequences can instruct the chemist in what metabolites the microorganism may be making, and the relationships to bioactivity and their relative proportions and importance can be aided with knockout mutation and other molecular tools.

6 DEVELOPMENT PROCESS OVERVIEW

Figure 8 shows the process for bringing a candidate from early stage discovery to launch. Due to the shorter time needed to develop a biopesticide compared to a chemical pesticide (3–5 years versus 11–12 years), many tasks are done in parallel rather than the sequential 'stage gate' development process typical of the development of a synthetic chemical. For example, natural product chemistry characterization will be conducted all through the development period because of the complexity, number and inter-relationships of the metabolites.

6.1 Process development

For our microbial products, we develop proprietary processes that increase the yield of both the microorganism and the active natural product compounds produced by the microorganism during fermentation. This chemistry-focused process development allows us to produce products that have superior performance, broader

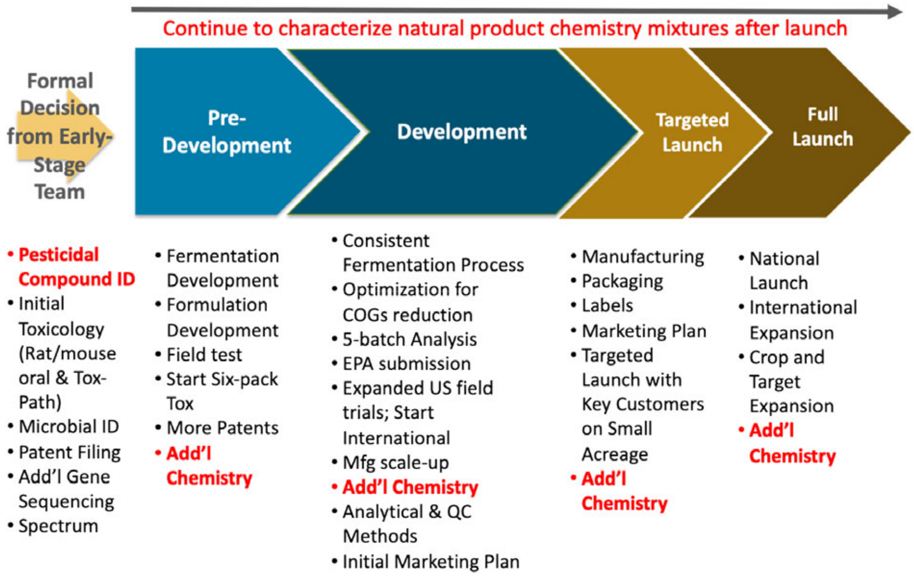


Figure 8. The process for moving a biopesticide candidate from early stage to commercial launch (Marrone Bio Innovations, unpublished).

spectrum, longer shelf life and ease of use compared to many living microbials, where processes focus on maximizing biomass rather than chemistry. We start with shake flasks, then 1-L vessels, followed by scale-up in progressively larger fermentation tanks. The manufacturing process goes through continuous improvement over time. We develop quality control methods based on the active natural product compounds rather than just the microorganisms or plant extracts. This approach results in a more consistent and effective product.

6.2 Formulation

We are able to develop proprietary water-soluble powder, liquid and granule formulations that allow us to tailor our products to customers' needs. The formulation development focuses on enhanced performance characteristics, such as efficacy, value, shelf life, suitability for organic agriculture requirements, water solubility, rain fastness, compatibility with other pesticides and sprayability. Formulation is critical to ensuring a natural pest management and plant health product's performance. Our understanding of the natural product chemistry allows us to develop formulations that maximize the effectiveness and stability of the compounds produced by the microorganisms or plants.

6.3 Field testing

We conduct numerous field trials for each product candidate that we develop. These field trials are conducted in small plots on commercial farms or research stations by our own field development specialists as well as private and public researchers to determine large-scale effectiveness, use rates, spray timing and crop safety. As the crop protection product candidate nears commercialization, we conduct demonstration trials on the farm. These trials are conducted with distributors but more often with influential growers in large blocks, typically 8–20 ha. These demonstrations comparing biopesticides in a program with the grower's traditional program are the best way to gain product adoption. The feedback from the grower is invaluable in adding new pests and crops to the label, and to feed back into research and development to make changes to formulations to better suit growers' needs.

6.4 Living versus dead microbials and plant extracts

There are currently hundreds of millions of dollars being invested (see Table 3) in companies developing living microorganisms and as a result there are few startups dedicated to products based on natural product compounds. One reason is that the regulatory process for living microorganisms is faster and has fewer requirements than non-living microorganisms (non-spore formers) and plant extracts. Despite the high biodegradability of the compounds, regulators tend to view these products more like chemical pesticides with additional toxicology requirements, even if the compounds are known to be of low risk and in food products.

7 HOW TO TEST AND USE BIOPESTICIDES BASED ON THEIR UNIQUE MODES OF ACTION

The single biggest barrier to the adoption and growth of biopesticides is the fact that they are often not tested based on their modes of action and are usually tested stand-alone rather than in rotations and tank mixes as is customary farmer practice with pesticides. For example, insecticide testing schemes in the greenhouse and field are often designed to test contact insecticides that kill in 48 h (Marrone Innovations, PG personal experience). There are many instances where biological insecticides are sprayed and rated like chemicals despite the fact that the most successful biopesticide in history is based on *Bacillus thuringiensis* (B.t.), which slowly kills small but not large caterpillar larvae after several days through ingestion but not by contact. One wonders if B.t. would ever make it to market today based on the narrow testing regimes that biopesticides are subject to. Test protocols, including observations of plant damage, yield and quality, should be incorporated into testing regimens. Another example is *Chromobacterium substugae* (Grandevo), where death of the insect pests may take as long as 7–10 days. Feeding stops in less than 1 min and reproduction is reduced. Care should be taken to use the proper water volume (too much reduces efficacy) and adjuvants. Some commonly used adjuvants reduced efficacy (http://cesantabarbara.ucanr.edu/files/187633.pdf). Well-designed and carefully implemented test protocols can maximize the efficacy of a product with a unique mode of action like this.

Table 5. Commercial microbial bioinsecticides

Active	Type	Pests controlled	Product examples	Manufacturer
Bacillus thuringiensis spp. *Aizawai*	Microbial, bacteria	Diamondback moth, armyworm	XenTari®, Agree®	Valent Bio., Certis USA
Bacillus thuringiensis spp. *Kurstaki*	Microbial, bacteria	A broad range of caterpillars	Dipel®, Deliver®, Foray®, Biobit®, Javelin®	Valent Bio., Certis USA
Chromobacterium subtsugae	Microbial, non-living bacteria	Broad range of sucking and chewing insects, mites and flies	Grandevo®	Marrone Bio Innovations
Burkholderia rinojensis	Microbial, dead bacteria	Broad range of sucking and chewing insects, mites and flies	Venerate®	Marrone Bio Innovations
Metarhizium anisopliae	Microbial, fungus	Thrips, mites, whiteflies	Met52®, GreenGuard®, Green Muscle®	Novozymes, BASF
Beauveria bassiana	Microbial, fungus	Sucking insects	Botanigard, several others	Bioworks, others
Apopka 97 strain of *Isaria fumosorosea*	Microbial, fungus	A broad range of sucking insects, mites and black vine weevil	PFR97®	Certis USA

Table 6. Commercial biochemical bioinsecticides

Active	Type	Pests controlled	Product examples	Manufacturer
Neem oil	Biochemical, soaps/fatty acids	A broad range of sucking insects, also fungi	Trilogy®	Certis USA
Azadiractin	Plant extract	A broad range of sucking and chewing insects	Aza-direct® (and others)	Gowan (and others)
Capsicum oleoresin extract; garlic, soybean oil	Plant extract, oils	Mites and a range of soft-bodied insects	Captiva® and Captiva® Prime	Gowan
Citrus oil solution	Plant extract	A broad range of sucking insects	Oroboost®	OroAgri
Crop oils	Paraffinic oil	Sucking insects	Stylet Oil®, Supreme Oil, others	Many
Chenopodium ambrosioides	Plant-derived terpenes	Sucking insects and mites	Requiem® (not organic)	Bayer Crop Science
Cyclotides	Butterfly pea extract	*Nezara*, *Helicoverpa*, mirids, whiteflies	Sero-X™	Innovate Ag
Spider venom peptides	Engineered into yeast cells, killed	Lepidoptera, some sucking insects	Spear™	Vestaron

Biologicals are frequently tested stand-alone, compared to the best chemical program of chemical rotations and tank mixes. It is more appropriate is to test each chemical and biological alone and then incorporate the biological into the program with the chemicals to show the benefit of the biological in the program. Often, you will see better efficacy and quality of the crop with the biological in the program. Even when the control is equal to the chemical program (no improvement in efficacy), the added benefits of resistance and residue management, shorter worker re-entry and zero-day pre-harvest intervals can make a compelling value proposition to growers.[14]

8 COMMERCIAL PRODUCT EXAMPLES

Examples of commercial products on the market for agriculture are shown in the tables below. As discussed earlier, due to the difficulty in discovering insecticidal natural products, there have been few novel products in recent years (only *Chromobacterium* and *Burkholderia*) (Table 5). A diversity of biochemical products is on the market based on neem, other plant extracts, essential plant oils and spider venom peptides (Table 6). New strains of *Beauveria* and *Metarhizium* continue to be found. There are many brands of *Trichoderma*-based biofungicides and only a few are listed here (Table 7). Certis recently commercialized a product from Montana State University's *Bacillus mycoides,* which is a strong plant defense activator rather than directly stopping spore germination like all of the other *Bacillus*-based biofungicides (Table 8). Bionematicides (Table 9) have had success as seed treatments stacked with chemicals for controlling plant parasitic nematodes on corn, soybeans and cotton. Some microbial products listed in the tables have the chemistry causing the pesticidal activity characterized and patented. Examples of these include a *Bacillus amyloliquefaciens* 713 biofungicide with novel lipopeptides marketed as Serenade®[15] and *Bacillus amyloliquefaciens (nakamurai)* F727 (Stargus® biofungicide) with different lipopeptides[16] (Table 8), *Chromobacterium subtsugae* PRAA4-1 (Grandevo®)[17] and *Burkholderia rinojensis* (Venerate® bioinsecticides and Majestene® bionematicide[18]) (Tables 5 and 9).

The chemistry of the plant-extracted products has more characterization because the regulatory agencies require knowledge of the main compounds causing the pesticidal activity.

An example of a product with a novel mode of action and novel chemistry is the microbial insecticide Grandevo (Table 5), based on the novel bacterial species *Chromobacterium subtsugae* PRAA4-1.[17] The bacteria produce several compounds of different

Table 7. Commercial non-*Bacillus* microbial biofungicides

Active	Type	Examples	Manufacturer
Trichoderma harzianum T-22	Microbial, fungi	RootShield® WP, PlantShield® HC	Bioworks
Trichoderma asperellum and *Trichoderma gamsii*	Microbial, fungi	BIO-TAM 2.0®	Isagro
Gliocladium virens	Microbial, fungi	SoilGard®	Certis USA
Coniothyrium minitans	Microbial, fungus	Contans® WG	Bayer
Pseudomonas chlororaphis strain AFS009	Microbial, bacteria	Howler™	AgBiome
Streptomyces lydicus	Microbial, actinomycete	Actinovate®, ActinoGrow®	Novozymes (Valent)

Table 8. *Bacillus*-based biofungicides

Active	Type	Product examples	Manufacturer
Bacillus amyloliquefaciens MBI600	Microbial, bacteria	Serifel®	BASF
Bacillus subtilis var. *amyloliquefaciens* FZB24	Microbial, bacteria	Taegro® 2 WP	Novozymes, distributed by Isagro USA
Bacillus subtilis IAB/BS03	Microbial, bacteria	Prevont®	Seipasa, distributed by Symagro
Bacillus subtilis (renamed *amyloliquefaciens*) 713	Microbial, bacteria	Serenade®, Cease®	Bayer
Bacillus amyloliquefaciens D747 (similar in lipopeptides to Serenade)	Microbial, bacteria	DoubleNickel 55®	Certis USA
Bacillus subtilus GB03	Microbial, bacteria	Companion®	Growth Products
Bacillus amyloliquefaciens ENV503 (genetically identical to *B. subtilis* GB03)	Microbial, bacteria	ENV503	Envera
Bacillus amyloliquefaciens (*nakamurai*) F727	Microbial, bacteria	Stargus®, Amplitude™	Marrone Bio Innovations
Bacillus mycoides isolate J	Microbial, bacteria	LifeGard® WG	Certis USA
Bacillus pumilus 2808	Microbial, bacteria	Sonata®	Bayer (Wilbur Ellis)

chemical classes in fermentation and when applied onto a crop as a wettable powder or granule (dead cells and compounds) repel pest insects, stop feeding in seconds and reduce adult insect fecundity. As mentioned earlier, the insects do not start dying until about 4 days, with peak mortality at 10 days. The bacteria produce purple pigment, violacein compounds that repel the pests and stop feeding (Fig. 8). The gut distress is caused by novel compounds we named chromamides (Fig. 8). The bacteria also produce at least six proteins, two of which are novel and synergize the chromamides in precise ratios.

Using this product like a knockdown insecticide of course may lead to disappointment. Therefore, education of users and key influencers in how to use the product early before pest populations increase is essential. In a program with other products, it is a good tool to improve overall pest management and population control, reduce residues and manage resistance.

Burkholderia rinojensis strain A396 was discovered in an insecticidal screen against beet armyworm, *Spodoptera exigua*, from a soil sample by Marrone Bio Innovations.[18] This bacterium produces insecticidal, nematicidal and herbicidal compounds depending on how it is fermented. Figure 9 shows the different compounds produced by this bacterium.

8.1 Bioherbicides

The technical difficulty in finding bioherbicides that can compete with the spectrum and price of chemical herbicides has left agriculture with a paucity of new herbicides based on natural products. Most products are targeted at organic agriculture because of their higher manufacturing cost. These products are typically short residual burndown products that require high volumes and work on the weeds' cuticles and membranes. Examples are clove, peanut, palm and orange (containing d-limonene) oils.

Table 9. Commercial microbial bionematicides

Active	Type	Product examples	Manufacturer
Purpureocillium lilacinus	Microbial, fungi	MeloCon®	Bayer Crop Science
Pochonia chlamydosporia	Microbial, fungi	KlamiC® and others	Several, ex-USA
Myrothecium verrucaria	Microbial, fungi	DiTera®	Valent BioSciences
Burkholderia rinojensis	Microbial, killed bacteria	Majestene®	Marrone Bio Innovations
Pasteuria nishizawae	Microbial, bacteria	Clariva® (seed treatment)	Syngenta
Bacillus firmus	Microbial, bacteria	Votivo® (seed treatment)	Bayer Crop Science
Saponins of *Quillaja saponaria*	Biochemical, plant extract	Nema-Q®	Brandt

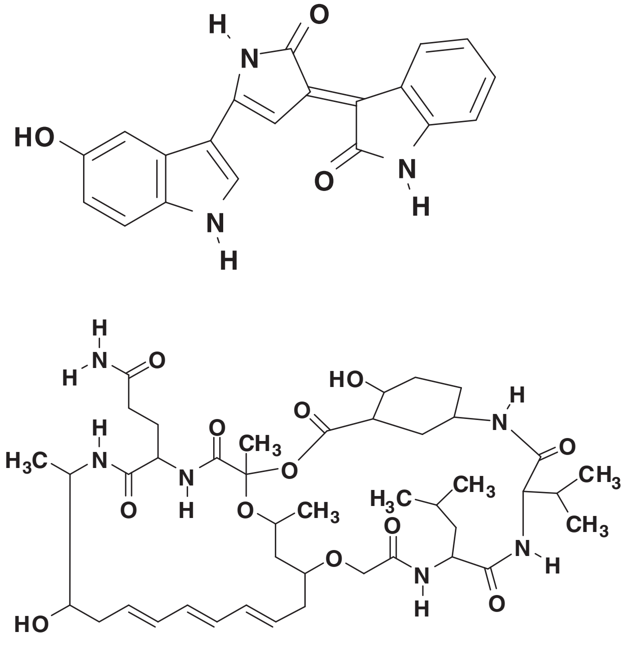


Figure 9. Violacein (top) and chromamide (bottom) produced by *Chromobacterium subtsugae*.

Other active ingredients include acetic acid (vinegar), FeHEDTA, NaCl, pelargonic acid, and caprylic and capric acids. One of the most successful organic products is Suppress®, which is based on caprylic and capric acids, but it still requires frequent, multiple applications and is only targeted at organic growers due to its cost.

Few companies have extensively screened microorganisms and plant extracts for herbicidal activity. Marrone Bio Innovations screened microbial supernatants in the herbicide and insecticide assay in parallel using a diet overlay assay against *Spodoptera exigua* (beet armyworm) and a glutamine synthetase (GS) assay.[13] GS was purified from a plant and an enzyme assay developed to screen supernatants to look for a mode of action like glufosinate which is a known GS inhibitor. Bialaphos is a natural herbicide produced by the bacteria *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*. Bialaphos is a protoxin that is non-toxic until metabolized in target weeds. When it is metabolized by the plant, the glutamic acid analog phosphinothricine (the natural version of the synthetic herbicide glufosinate) is released, which inhibits GS, causing ammonia build-up in the cell.[19]

The novel species of *Burkholderi*a (*rinojensis*) was active in both the herbicide and insecticide screens. Once we found activity on insects, we tested root knot nematode and found that it killed them also. The chemistry has some bioactivity overlap, but for the most part there are insecticidal, nematicidal and herbicidal distinct active fractions. We found that we could develop a different fermentation process to replace insecticidal activity with herbicidal activity. The herbicidal active fractions with molecular weights of 540 and 519 are shown in Fig. 10. MW 519 is spliceostatin C and MW 540 is related to romadepsin, an anticancer drug. In work conducted by Stephen Duke's laboratory, MW540, which occurs in the supernatant of the *Burkholderia* fermentation process, is a histone deacetylase inhibitor. Spliceostatin C, a known compound in anticancer research, is extremely toxic to *Amaranthus* weed species, which have become very problematic in glyphosate-resistant crops. It disrupts the RNA splicing of several genes during transcription by interfering with the spliceosome. This potent phytotoxin occurs in the supernatant of the *B. rinojensis* herbicide fermentation process. Compared to known commercial herbicides, spliceostatin C is very potent (Table 10).

9 COMPARISON OF BUSINESS MODELS: CAPITAL LIGHT VERSUS HEAVY CAPITAL OF LARGE AGRICHEMICAL COMPANIES

One of the misunderstandings about biopesticides is the different business model compared to the business model for developing and launching a synthetic chemical pesticide. Because of the long time (10–12 years) and upfront capital cost (more than US$280 million) for developing a chemical, by the time a chemical pesticide reaches the market, there are thousands of field trials and demos, the manufacturing process and formulations are perfected and global regulatory approvals are pending. Therefore, when a chemical is launched, it is launched big. Peak sales are expected in 3–5 years. Companies need to quickly maximize revenues before expiration of any patents protecting the chemical pesticides.

For a biopesticide, often developed by smaller companies without the deep pockets of multi-billion-dollar companies, there is a different 'capital-lite' model applied, which could be called the 'innovate at speed' or 'agile innovation' model. This model is capital efficient and 'fund as you go'. Because of the 70-year history of safety and low risk of biopesticides, short development time and favorable regulatory process in the USA, a small company can enter the market with version 1.0 biopesticide and place the product in a controlled fashion with a few early adopter grower customers. This provides valuable early insight from customers and feeds back into research and development for the next generation product, version 2.0, allowing rapid and continued innovation. Because an early version may have only a US label with a few crops and uses, peak sales do not occur in 3 years, but will take longer (5+ years), as more uses, crops and international approvals are achieved over time. Neither the capital-intensive model nor the capital-lite 'agile innovation' model are 'right' or 'correct'. They are simply different, and understanding this will lead to better expectations for biopesticides when they enter the market. Many growers love to get access to a product early and there is a great amount of information gained from their experiences with it. The 'agile innovation' model is used widely in places like Silicon Valley. Consumers are accustomed to continually having new versions released of their iPhone hardware and software (helping the companies de-bug the new version). Yes, imagine if a biopesticide had US$280 million in research and development (instead of US$3–7 million) behind it before being launched? It would be a very different product! But then, biopesticide innovation would decrease because only large companies could afford the cost. Figure 11 summarizes the different business models and ways to develop a biopesticide versus a chemical product.

10 BARRIERS TO ADOPTION OF BIOPESTICIDES

10.1 Perceptions persist about efficacy and cost

There is a lack of awareness and understanding of biopesticides by agronomists, growers, crop consultants and key influencers such as university and government researchers. As such, they are pigeon-holed into 'organic only.' Biopesticides are often not tested or used properly based on their unique modes of action. It is critical to educate the sales force, users and key influencers

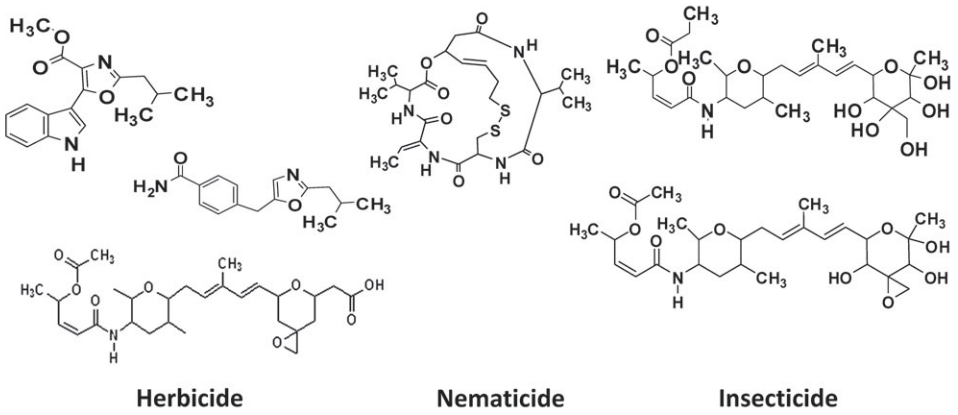


Figure 10. Some of the compounds produced by *Burkholderia rinojensis* A396.

Table 10. Comparative potencies (amount needed for effective control) of different herbicides in comparison to spliceostatin C

Product	Grams per acre of active ingredient
MBI-014 (spliceostatin C)	0.3
Raptor (imazamox)	16.2
Roundup (glyphosate)	109
Sethoxydim	182
Prowl (pendimethalin)	299
Rely (glufosinate)	348
Atrazine	907
2,4-Db	971
Suppress (caprylic and capric acids)	4486 (mL)

Data from Marrone Bio Innovations.

to use the product early before pest populations increase. Or if pest populations are already high, start with another insecticide that has contact activity or more knockdown effects, followed by a product such as Grandevo. Incorporated into a program, biopesticides can be used in conventional programs for resistance and residue management, and to increase efficacy of chemicals.

10.2 Read the Label

Other things to watch for when integrating biopesticides include water pH, mixing order in the tank and the choice of adjuvant, which can increase or decrease efficacy. In addition, it is quite common to hear a grower or consultant say they used a biopesticide for the first time when nothing else would work, including chemical pesticides. 'I tried everything but the kitchen sink, so I think I'll try a biopesticide.' This is exactly the wrong time to try biopesticides. One bad experience can linger for many years.

10.3 Formulation

A formulation can make or break a biopesticide. Formulation innovation can transform biopesticides with new inerts and formulations to extend field residual life and improve consistency. A number of companies have formed to improve biopesticide formulation (e.g. Crop Enhancement, Terramera). To take advantage of the synergies of biologicals and chemicals, companies are developing formulation pre-mixtures of chemicals and biopesticides such as StK's Regev®, and several microbial + chemical stacked seed treatments, as described previously.

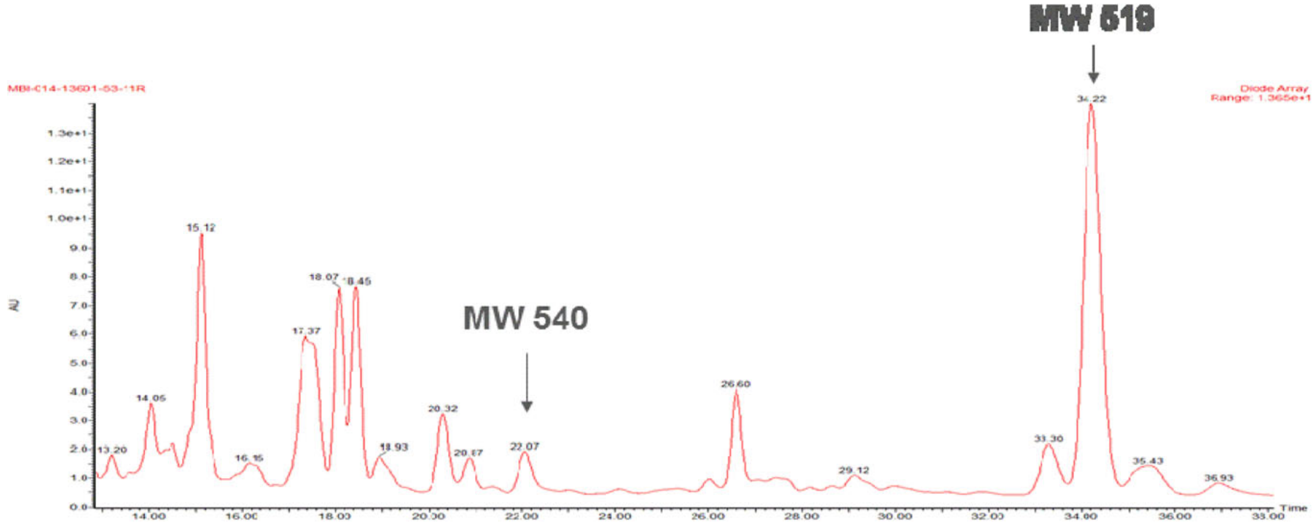


Figure 11. Profile of the key herbicidal compounds produced by *Burkholderia rinojensis* A396.

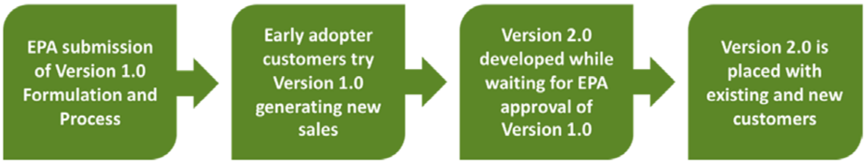


Figure 12. 'Innovate at speed' business model for biopesticide market launch.

10.4 Getting through the distribution channel

Small startup biopesticide companies typically focus on getting their first product through the regulatory process. This is important but then what? The go-to-market strategy is fraught with challenges. The agrichemical market is very crowded and hyper-competitive. For a small one-product company to get the attention of a distributor who is the gatekeeper for the relationship with the grower in the USA is not easy. One strategy is to continue to innovate by developing a full portfolio of products across the full range of customer needs (insect, nematode, plant disease and weed control). A distributor would like to continually provide something new that meets unmet customer needs. Companies bringing products to market, whether chemical or biological, see faster adoption when filling an unmet need. For example, faster adoption may occur when entering with a new bionematicide, an effective bioherbicide (especially for organic production) or an effective and safer fumigant. This compares to coming to market with another similar biofungicide for powdery mildew and leaf spots, which is a crowded market segment.

Another possibility is to partner with a large agrichemical company to do the sales and marketing of your biopesticide product. This takes careful consideration since you will sacrifice profit margin by having another entity along the chain to the farmer. In theory, higher volumes should make up for lost margin, but many small companies have found that this is not always the case. For large-scale row crops, partnering may be the best model as it takes a large sales force to access hundreds of millions of hectares of corn and soybeans.

Some new venture capital-backed biostimulant companies are testing a new model that bypasses the distributor by going direct to the grower. Time will tell whether status quo will continue, or disruptive, innovative new entrants with roots outside of agriculture will change the difficulty of accessing the grower via distribution.

11 THE BIOPESTICIDE FUTURE

'Big data' is being applied on the farm to increase yields by understanding soil types, soil and crop water, crop varietal effects, weather and microclimates, and the microbiome, among others. What has lagged, however, is the application of 'big data' to pest management. While there are certainly some pest and disease-specific degree-day models developed at universities and government institutes, 'big data' and precision farming have not been as extensively used in local and regional predictions of pest and pathogen populations for more accurate spraying in time and space. Fungicides are still largely applied on a calendar basis. Because timing of biopesticide application is so critical based on their unique modes of action and need to spray early, better scouting and pest/disease population prediction tools will make biopesticide application timing more efficient and effective. Vision/video and drone-based systems to record pest populations in the field in real-time can reduce or eliminate manual scouting. Infrared sensors can assess how well a pesticide application has reduced pest populations. Sensor-triggered spraying with variable rates depending on pest population is already here with companies like Semios, where pheromones are released via a sensor-controlled system based on moth populations detected by a vision-based trap. The future is technology, and we are truly just beginning to marry 'the connected farm' with pest management. Biopesticide adoption will increase substantially as technology, data and pest management are integrated.

To summarize the state of biopesticides and how to increase their adoption:

- Biopesticides have become better over time: they are now more science-based, have better formulations and manufacturing processes, with novel species/strains.
- Living microbes are very popular, with most investment dollars going into startups with live microbes, soil health and synthetic biology of microorganisms.
- Most new companies do not address the chemistry behind their products (except to use gene sequencing to understand what the microbe might be making) because of technical difficulty, expense and increased regulatory scrutiny.
- Increasing grower receptivity to biologicals, but unsure how to use them.
- More education and training on how the products work and how to integrate them into Integrated pest management/crop production programs,a nd understand their unique modes of action.
- Government investment is needed to fund development of holistic ecologically based integrated programs of biopesticides along with other tools, cultural practices such as crop rotation and cover crops, improved crop varieties and soil health.
- Conduct on-farm demonstrations: block with the biological in the program compared to grower's traditional program. Not just stand-alone trials compared to the chemical program.

12 RESOURCES AND TRADE ASSOCIATIONS

12.1 The Bioproducts Industry Alliance

The Bioproducts Industry Alliance (BPIA, http://www.bpia.org), created in 2000, is dedicated to fostering adoption of biopesticide technology through increased awareness about their effectiveness and full range of benefits to a progressive pest management program. BPIA members typically meet twice per year, rotating locations in Washington, DC, Sacramento, CA and Ottawa, Canada. Committees. The BPIA Regulatory and Government Affairs Committees are active in insuring that regulations remain transparent and meet statutory timelines for approvals.

12.2 The International Biocontrol Manufacturers' Association (IBMA)

The International Biocontrol Manufacturers' Association (IBMA, http://www.ibma-global.org) is the worldwide association of biocontrol industries producing microorganisms, macroorganisms, semiochemicals and natural pesticides for plant protection and public health. The IBMA was created in 1995 to represent the views of these biological control producers, who are mainly small companies with limited resources: manufacturers, research organizations, extension services, consultants, distributors, all contribute to the development of biocontrol and participate in IBMA activities. The IBMA actively seeks to form a global federation of like-minded regional associations and has already formed a working link with the BPIA in North America. The IBMA holds an annual member meeting in October in Basel, Switzerland.

12.3 IR-4 (USDA program housed at Rutgers University)

The primary objective of the IR-4 Biopesticide and Organic Support Program (http://ir4.rutgers.edu/biopesticides.html) is to further the development and registration of biopesticides for use in pest management systems for specialty crops or for minor uses on major crops. IR-4 has an efficacy grant program that researchers can apply to for funds to do early field trials with biopesticides and also to demonstrate their performance in IPM programs. IR-4 has a searchable biopesticide label database. Through its many years of registering biopesticides and supporting biopesticides through its efficacy and other educational initiatives, IR-4 has been instrumental helping educate users and researchers about the best use of biopesticides and their benefits in IPM programs. IR-4 has a close collaboration with BPIA.[20]

13 CONCLUSIONS

Biopesticides continue to grow at a pace that exceeds chemical pesticides and when incorporated into pest management programs can provide benefits that customers are increasingly recognizing, such as residue and resistance management, shorter worker re-entry and low risk to beneficial organisms, including honeybees. Most important, however, is that biopesticides can make conventional programs better, increasing yield and quality compared to chemical-only programs. Biopesticides meet consumer demands for health and wellness. GM crops and chemical pesticides currently dominate pest management programs and are largely seen as essential requirements to feed the world. Restrictions on chemical pesticides are expected to continue and resistance has become a factor in the deployment and sustainability of GM crops. As such, biopesticides can be the third leg of technology inputs, and over time can increase the output, durability and sustainability of IPM programs.

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Perspective

This article reviews the status, history and future of natural products used as biological pesticides, which are the most rapidly growing agricultural inputs category.

Pesticidal natural products – status and future potential

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