Plant Life
by Ben Jardine

I.

The wide-brimming leaves of *Nicotiana benthamiana*—"tobacco" to the layman, from the nightshade family—may hold the key to eradicating some of the most pressing threats to life around the world. Originating in areas of Australia and South America, the cash crop arrived on Europe's shores in the midst of colonial expansion some five hundred years ago. Since then it has served as a cash crop, a stimulant, and a weight loss drug (the word "nicotine" originates from a story in which French ambassador to Portugal, Jean Nicot, prescribed medicinal tobacco to Catherine de Medici), until the mid-1990s when studies showed tobacco was a health hazard. In 2001, tobacco was named the second most preventable cause of death by the World Health Organization, after hypertension. However, despite more and more recent research showing tobacco's health risks, the plant is now in a position to actually *save* lives.

"There are two types of vaccines on the US market today," explains Timmy Hardman, a researcher at Pitzer College's Ferre/Marquet Vaccine Research Center (FMVRC). "One is made with animal cell cultures, and the other [is] made from chicken eggs. Both of these are super expensive, and both require advanced refrigeration to transport, cuz they're potentially harmful viruses, y'know? Animal based production is just difficult at every stage of the process." At a foundational level, vaccines operate by tricking the body's immune system into thinking it is under attack from a specific pathogen. Current vaccine production methods use attenuated versions of the virus which pose no real threat to the immune system, because of their weakened state. Plant-based

vaccine production uses no genetic material from the virus being fought—it only utilizes the parts of the virus that the immune system recognizes: antibody binding sites. By displaying these sites on a "scaffolding" protein, the immune system takes defense measures against a sheep in wolf's clothing.

Faced with an increasing global population and skyrocketing vaccine prices, Timmy is one of several researchers worldwide looking to our chlorophyll-filled co-inhabitants for answers in countering these issues. And *N. benthamiana* has been receiving the most attention.

The logic behind *N. benthamiana* in plant-based vaccine research is twofold. Firstly, the plant has a high susceptibility to plant pathogens (in fact, the first ever discovered virus, tobacco mosaic (TMV), was found in the leaves of *N. benthamiana* in 1886), which means that grafting a pathogen onto the plant is relatively simple. Secondly, TMV-infected leaves are more fragile than their healthy counterparts, allowing for easier extraction of the virus following infection. To put it another way, the infected plant is the well-seasoned road warrior you would run into in a plant virus bar: growth stunted, with mottled leaves, but full of stories about Charles Darwin's *Beagle* and its trials in pharming. The virus sits at the center of a global effort to wipe out viruses on animal-based, zoonotic (affecting both humans and animals), and human levels—all at affordable prices immune to Big Pharmaceutical influence.

To the *N. benthamiana,* TMV is potentially life threatening—but to all mammalian life, TMV is an elixir. And an elixir that, seemingly, walks the walk.

In fact, most of the "walk" is relatively simple to comprehend: first, an *N. benthamiana* sapling is exposed to a DNA vector that codes for the production of the

virus-to-be-vaccinated, by being submerged in a cup of *agrobacterium* (gene transfer bacteria). This creates a vacuum on the surface of the cup, which allows for infiltration of the vector through plant pores. The plant is then transferred to a growth chamber for one to two weeks while the virus proliferates. Over time, the DNA of the virus merges with the DNA of the plant, and the virus takes host of the tobacco plant. Then, once that plant grows to an appropriate size, the virus can be isolated from the rest of the plant and extracted through centrifugal force.

If all this sounds fascinating, that's because it's only been in the past few years that this work has had the results to prove its effectiveness. A senior at Pitzer, Timmy has been working on *N. benthamiana*-based vaccines for around three years now. His thesis, which explored alternatives in vaccine production, touched on some research he had conducted with the FMVRC in rural Botswana. Stationed in Gaborone, at the University there, Timmy worked with a team of four others to develop a plant-based vaccine for Lumpy Skin Disease (LSD)—a virus responsible for wiping out cattle populations of epidemic proportions. More recently, Timmy has been working on a vaccine for rabies.

When asked why Botswana, of all places, was ripe for plant-based vaccine research, Timmy points to a vastly open market and another, more pressing, social issue there. "In Botswana cows outnumber people two to one, and when farmers' cows get sick with LSD, they can't afford to pay for the expensive vaccines offered by pharmaceutical companies. And these companies haven't pursued R&D into the capabilities of plants, and so there's just this gaping market there."

A very big (and global) market indeed. As Timmy has explained, animal-based vaccines are costly to produce, to maintain, and to distribute. For these reasons, many companies in the US and abroad are beginning to pursue more substantial alternatives.

The key figure in all of this is Dean of Research at Keck Graduate Institute (KGI) FMVRC Director, and Timmy's thesis advisor, Dr. Larry Grill. With several decades of experience in the biotech industry, Professor Grill is part of the vanguard that has made it their life's work to pursue plant-based alternatives in vaccine synthesis. In 1987, he maxxed out his credit cards to start Large Scale Biology Corporation, a biotech firm pioneering plant-based medicine, which, over the course of 18 years, soon grew to 300 people and raised over $350 million through venture capitalist investment. Grill served as Chief Science Officer there until 2006, when sourcing funds became too difficult and the company was forced to go out of business. This is a common theme for companies that are heavily involved in plant-based vaccine research. Because the Food and Drug Administration (FDA) has never approved the use of plant-based medical proteins in treating disease, no US-based plant research company can get federal funding. Nowadays, Professor Grill heads the collaborative effort between the respective vaccine institutes of Pitzer (which receives no federal funds for vaccine research) and the University of Botswana (which does).

Professor Grill has a unique lens with which he approaches plants and their potential, which comes from a lifetime of experience in the field: "When I walk through farmer's markets, I see that plant has yellow mosaic, that plant has papaya ringspot. I see the plant viruses that are there, that we eat all the time." Plant viruses, like TMV, do not pose a risk to humans, Grill explains, "but if you were to inject it into your blood, your

immune system would make tremendous amounts of antibodies against it, because it is a virus and your immune system recognizes it as a virus, and it really reacts to it. In a way you could say it's wasted energy, because the virus can't cause disease in humans anyway. It doesn't have any of the abilities to do it."

Here is where the future becomes dazzling and all-too-possible: if a protein from the rabies virus is extracted and "decorated" onto the TMV, the human/mammalian body will produce an immune response to both viruses. However, when the body is exposed to the rabies virus later on, it will be able to fight it as if it had been exposed before—which, it had, except the first time it was disguised as a plant virus. Non-harmful plant viruses can act as surrogates for immunization against, in theory, nearly any potentially life-threatening virus.

II.

Big Five pharmaceutical companies worldwide, ranked in order of 2014 market control[1]:

1) Merck & Co., in Kenliworth, New Jersey: $6.25B revenue
2) Sanofi Pastuer SA, in Lyon, France: $5.85B
3) GlaxoSmithKline PLC, in Brentford, England: $5.26B
4) Pfizer, Inc., in New York City, New York: $4.48B
5) Novartis AG, in Basel, Switzerland: $1.53B

Five companies control the distribution of vaccines globally. Keeping in mind that these companies all deal with the pricey animal-based vaccine, it must be said that these companies spend disproportionate amounts of money on marketing rather than research and development. In a recent article (2015)[2], the Washington Post reported that Big

Pharma spent roughly half of what they spent on marketing, specifically to doctors, on R&D pursuits. These figures, staggering as they are, really reflect an inherent flaw in how Big Pharma approaches treating medicine. Given their current track record, it seems unlikely these companies will pursue alternatives in vaccine production—which leaves the market for plant-based alternatives wide open. So, why won't these companies pursue cheaper alternatives?

In the world of Big Pharma, "there is no incentive to make an inexpensive vaccine," explains Dr. Grill, offering up anecdotes: "If I were to talk to one of the Big Pharma companies and say, 'hey, I can make this vaccine for $1'—let's say—compared to your $45 a dose. They're gonna say, 'we don't want that'. If it costs a dollar to make the vaccine, and you charge five times what it costs to make, that's four dollars profit. If they make it for $40 and sell it for five times the price, that's a whole lot more money."

So in what direction can plant-based vaccines move, with the most powerful companies in the world uninterested in what is possible?

Over the past few years, philanthropic efforts have helped push vaccine research into new realms. In 2005, Warren Buffett gifted ten million Berkshire Hathaway shares to the Bill & Melinda Gates Foundation, to be spread over several years, worth around a billion dollars a year. The goal: to help "support the innovation needed to develop new vaccines and new delivery technologies and approaches"[3].

And there is some encouragement from other venture capitalist investments—like those who invested in Grill's biotech firm. Funding has been a learning game for Grill: "I have a meeting with the Batswana government, and they are kind of saying that they'll go 50/50 in on it. And I have other sources, mostly philanthropic."

But the real key to more funding, it seems, is to cast awareness wide. In 2010, the global health community proclaimed 2011-2020 the "Decade of Vaccines" in an effort to extend immunization to all people, regardless of geographical location, identity, or economic prosperity. With more research into vaccines for animals (drug trials for the LSD vaccine are currently underway), the effectiveness of plant-based vaccines will begin to speak for itself. And as social fears begin to quell, more people around the world will see the overwhelming benefits of using tobacco plants to make vaccines. Grill approaches the global use of plant-based vaccines in three stages: the first stage is to show how plant-based vaccines work on animals—the LSD vaccine, in Botswana is an example of this first stage. The second stage is to then show how they work on zoonotic diseases, which infect both humans and animals (rabies). The third stage, yet to be approached, is to show how plant-based vaccines work on humans.

"The one that we're next actually going after, and we may get started on that later this year, is the HPV," he says. Human papillomavirus (HPV) is the most frequently sexually transmitted disease in the world and has been shown to lead to cervical, skin, and genital cancers. In 2012, the World Health Organization reported 528,000 new cases and around 266,000 cervical cancer related deaths. 85% of these reported cases occurred in the developing world.

"We have very good vaccines for those," Grill explains. "But the problem is this: if you live in the US, your insurance company will pay for a vaccine. If you don't have insurance it costs between $140 and $150 a dose, and you need three doses. That's $450. But, if you go to Africa where: Number 1, they don't have insurance; and Number 2, they don't have $450—the rate of HPV in Africa is huge. And that means that the death [rate]

from cervical cancer is huge. And there's nothing we can do about it. The cost is just too huge, so if we can make a really cheap vaccine, that's going to cost a buck or something like that, then we can actually stop cervical cancer in Africa, and that's a known fatality. So we already know that one is approachable."

All this speaks to a greater philosophical question: just because something has been done before, and has been proved effective, does not mean it is the end-all solution. "We have to be open to change and understand imperfections in the systems that exist already," Timmy concludes. "That's how we improve as a species. That's how we adapt to our surroundings and our environment."

Massive changes in global health are just around the corner, and the potential is almost unbearable. Tobacco plants, manipulated genetically to serve the will of humankind, represent an impossible value that no other plant has ever come close to embodying. They really are a "cash" crop unlike any other.

---

[1] Caceras, Marco. "Merck Leads World's Top Vaccine Makers, Novartis Out." *The Vaccine Reaction*. N.p., 13 Oct. 2016. Web. 11 Apr. 2017.

[2] Swanson, Ana. "Big Pharmaceutical Companies Are Spending Far More on Marketing than Research." *The Washington Post*. WP Company, 11 Feb. 2015.

[3] "Vaccine Delivery: Strategy Overview." *Gates Foundation*. Bill & Melinda Gates Foundation