UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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INARI AGRICULTURE, INC.

Petitioner,

v.

CORTEVA AGRISCIENCE LLC,

Patent Owner.

_____

PGR2023-00022

U.S. Patent No. 11,371,055

DECLARATION OF DR. ARON SILVERSTONE, Ph.D.

TABLE OF CONTENTS

I. INTRODUCTION 1

II. BACKGROUND AND QUALIFICATIONS 2

III. LEVEL OF ORDINARY SKILL IN THE ART 6

IV. MATERIALS CONSIDERED AND RELIED UPON 7

V. LEGAL STANDARDS 7

A. Written Description Requirement 8

B. Enablement Requirement 9

VI. DESCRIPTION OF THE RELEVANT FIELD AND THE RELEVANT TIMEFRAME 11

VII. OVERVIEW OF THE '055 PATENT 12

A. Subject Matter Overview 12

B. Prosecution History 15

VIII. CLAIMS 1-33 LACK PROPER WRITTEN DESCRIPTION SUPPORT AND ARE NOT ENABLED 15

A. Claims 1-33 Lack Sufficient Written Description that Shows that Patent Owner was in Possession of the Claimed Invention 15

1. The AAD-12 Structure Disclosed in the '055 Patent is Insufficient to Describe a Genus 15

2. The Examples Disclosed in the Patent are not Reasonable to Support the Broad Genus 21

B. Significantly More Support Would be Necessary for the Broad Genus Claimed 23

1. Extensive and Undue Experimentation is Required to Practice the Full Scope of the Claimed Invention 25

IX. CONCLUSION 42

I, Aron Silverstone, Ph.D., hereby declare under penalty of perjury:

I. INTRODUCTION

1. My name is Aron Silverstone, and I have been retained by counsel for Petitioner Inari Agriculture, Inc. ("Inari" or "Petitioner") as an expert witness to provide assistance regarding U.S. Patent No. 11,371,055 (the "'055 Patent"), which I understand has been designated as Ex. 1001. Specifically, I have been asked to consider the validity of claims 1-33 of the '055 Patent (the "Challenged Claims") in view of prior art, written description and enablement requirements, and the understanding of a person of ordinary skill in the art at the time of the invention as it relates to the '055 Patent. I have personal knowledge of the facts and opinions set forth in this declaration and believe them to be true. If called upon to do so, I would testify competently thereto.

2. I am being compensated for my time at my standard consulting rate. I am also being reimbursed for expenses that I incur during the course of this work. My compensation is not contingent upon the results of my study, the substance of my expert opinions, or the outcome of any proceeding involving the challenged claims. I have no financial interest in the outcome of this matter or on the pending litigation between Petitioner and Patent Owner.

3. My analysis here is based on my years of education, research and experience, as well as my investigation and study of relevant materials, including those cited herein.

4. I may rely upon these materials, my knowledge and experience, and/or additional materials to rebut arguments raised by the Patent Owner. Further, I may also consider additional documents and information in forming any necessary opinions, including documents that may not yet have been provided to me.

5. My analysis of the materials produced in this proceeding is ongoing and I will continue to review any new material as it is provided. This declaration represents only those opinions I have formed to date. I reserve the right to revise, supplement, and/or amend my expert opinions stated herein based on new information and on my continuing analysis of the materials already provided.

II. BACKGROUND AND QUALIFICATIONS

6. I am an expert in the fields of plant physiology, biochemistry, molecular biology and genetics. I have studied, taught, practiced, and researched this field for over 35 years. I have summarized in this section my educational background, work experience, and other relevant qualifications. Attached hereto as **Appendix A**, is a true and correct copy of my curriculum vitae describing my background and experience.

7. I earned my Bachelor of Arts degree in Biology from Harvard University in in 1987. In 1994, I earned my Doctor of Philosophy degree in Plant Physiology from University of California, Davis, with my dissertation being on "The Physiology and Biochemistry of Tryptophan Malonylation in Higher Plants."

8. During my dissertation research, I investigated the biosynthetic pathways of the plant hormones auxin and ethylene, and I identified novel amino acid metabolism reactions.

9. After obtaining my Doctor of Philosophy degree, I worked as a post-doctoral fellow in the Botany Department of Duke University I conducted research on the biosynthesis and signal response pathway of the plant hormone gibberellin.

10. In June of 1999, I joined Syngenta Biotechnology, Inc. as a Staff Scientist I. In this position, I designed and built constructs to express a sweet protein in corn and analyzed transgenic plants. In February of 2001, I was promoted to a Staff Scientist II position. In this role, I discovered and validated seed-specific promoters. In June of 2003, I was promoted to Project Leader. I lead teams that developed novel starch degrading enzymes and expressed them in transgenic corn. I also managed the development of a new transgenic product expressing a high temperature amylase in corn for the corn starch-to-ethanol industry. Another team I led generated the second generation of Golden Rice, rice that produces beta-

carotene. This required expression and analysis of novel carotenoid biosynthetic enzymes in rice.

11. In April of 2007, I received a promotion to Senior Project Leader. While in this position, I developed technologies to enhance the ability to express heterologous proteins in plants.

12. In July of 2010, I received a promotion to Global Project Manager for Soybean Traits in Syngenta Seeds, LLC. While in this position, I led a multifunctional team to develop a novel herbicide-tolerant soybean. The work included lab-based assays, field studies, regulatory strategy, product safety studies, marketing, and IP. I also led a multifunctional team to develop a novel insect control transgenic trait stack in soybean. The activities included coordinating lab studies, field activities, IP, product safety studies and regulatory strategy, and marketing. In June 2014, I became a Product Biology Lead for Abiotic Stress Management, North America for Syngenta Crop Protection, LLC. My work in this position entailed overseeing the field trial program to test chemical products for performance in mitigating yield loss from abiotic stresses. It also included designing trials and testing methods to identify crop enhancement benefits from fungicides. In January 2019, I became a Senior Team Lead in Product Safety Operations. As Senior Team Lead, I led a team of specialists supporting 100+ Product Safety scientists for budgeting, outsourcing, document management, knowledge management and lab

management. In November 2020, I became a Technical Lead in Human Safety Residue Science. While in this position, I led a team of scientists overseeing residue studies for regulatory submissions in US and Canada. I also supported the product development of new active ingredients and the expansion of the use of registered pesticides.

13. In February 2023, I accepted a position as a Scientific Consultant at AgStone Research, LLC. My work in this position, has thus far entailed to consult on scientific strategy in the field of biotechnology for agriculture.

14. I have authored and co-authored over 17 journal publications, conference proceedings, technical papers, patents, book chapters, and delivered over 100 technical presentations in a broad array of subjects specific to my research programs as well as on the subject of plant biotechnology and crop improvement. I have also served as a guest lecturer on plant biotechnology at Duke University since 1997. A complete list of my publications and selected list of invited talks is also contained in my *curriculum vitae*.

15. I have also received a number of awards and recognitions, including the Rising Sun Award at the 16th International Conference on Plant Growth Substances among others, which are listed in my *curriculum vitae*.

III. LEVEL OF ORDINARY SKILL IN THE ART

16. In rendering the opinions set forth in this declaration, I was asked to consider the patent claims and the prior art through the eyes of one of ordinary skill at the time of the claimed invention, which I understand may be asserted to be March 24, 2017—the filing date of the application that issued as the '055 patent, and the earliest priority date to which the claims of the issued patent are entitled. I understand that the factors considered in determining the ordinary level of skill in a field of art include the level of education and experience of persons working in the field; the types of problems encountered in the field; the teachings of the prior art, and the sophistication of the technology at the time of the claimed invention. I understand that one of ordinary skill is not a specific real individual, but rather is a hypothetical individual having the qualities reflected by the factors above. I understand that one of ordinary skill would also have knowledge from the teachings of the prior art, including the art cited below.

17. Taking these factors into consideration, on or before March 24, 2017, one of ordinary skill relating to the technology of the '055 patent would have had a Ph.D. in biochemistry, cell biology, genetics, or a related field, and 3-5 years of experience in the design or development of transgenic plants including genomics, proteomics, biochemistry, plant transformation and crop science, or the equivalent. Additional postdoctoral work could substitute for professional experience.

18. Before March 24, 2017, my level of skill in the art was at least that of one of ordinary skill. I am qualified to provide opinions concerning what one of ordinary skill would have known and understood at that time, and my analysis and conclusions herein are from the perspective of one of ordinary skill as of that date.

IV. MATERIALS CONSIDERED AND RELIED UPON

19. In reaching the conclusions described in this declaration, I have relied on the '055 Patent, the prosecution history of the '055 Patent, the prior art, and other documents and materials cited herein. For ease of reference, the full list of documents that I have considered is included in **Appendix B**.

20. These materials comprise patents, related documents, and printed publications. Each of these materials is a type of document that experts in my field would have reasonably relied upon when forming their opinions.

21. I have also relied on my education, training, research, knowledge, and personal and professional experience in the relevant technologies and systems that were already in use prior to, and within the timeframe of the earliest priority date of the claimed subject matter in the '055 Patent, which is March 24, 2017.

V. LEGAL STANDARDS

22. I am not a lawyer and do not provide any legal opinions, but I have been advised that certain legal standards are to be applied by technical experts in forming

opinions regarding meaning and validity of patent claims. I have applied the legal standards described below, which were provided to me by counsel for Petitioner.

A. Written Description Requirement

23. I have been informed that the statutory basis for the written description requirement is found in 35 U.S.C. §112(a), which provides in relevant part:

> (a) IN GENERAL.—The specification shall contain a written description of the invention…

24. I have been informed by counsel that a claim in a granted patent must be sufficiently supported by the disclosure in the patent's specification, read in the context of what a person of ordinary skill in the art would have known at the time of the claimed invention. I understand that the basic inquiry for written description is whether the specification provides sufficient information for the person or ordinary skill to recognize that the named inventors possessed the full scope of the claimed invention.

25. I have been informed by counsel that the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species of the genus by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant

was in possession of the claimed genus. I also understand that the disclosure must adequately reflect the structural diversity of the claimed genus, either through the disclosure of sufficient species that are representative of the full variety or scope of the genus, or by the establishment of a reasonable structure-function correlation. I understand that such correlations may be established by the inventor as described in the specification, or they may be known in the art at the time of the filing date.

B. Enablement Requirement

26. I have been informed that the statutory basis for the enablement requirement, which is an additional requirement beyond the written description requirement, is found in 35 U.S.C. §112(a), which provides in relevant part:

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same…

I have been informed that a claimed invention is not enabled under 35 U.S.C. §112(a) if the specification does not teach those of ordinary skill in the art how to make and use the invention as broadly as it is claimed, without undue experimentation.

27. I have been informed that the full scope of the claimed invention must be enabled.

28. I have been informed that the assessment of undue experimentation is based on the state of the art as of the earliest filing date of the applications on which

the ‘055 Patent claims priority. In this case, I have been informed that date is March 24, 2017, the filing date of nonprovisional application 15/468,494.

29. I have been informed that a finding of undue experimentation must consider multiple factors to determine if there is sufficient evidence to support such a determination. I have been informed that these factors, which are referred to as Wands factors, include (1) the nature of the invention, (2) the relative skill of those in the art, (3) the breadth of the claims, (4) the presence or absence of working samples, (5) the amount of direction or guidance presented, (6), the state of the prior art, (7) the predictability or unpredictability of the art, and (8) the quantity of experimentation necessary.

30. I have been informed that to determine whether there is a reasonable correlation between the scope of the claims and the scope of enablement, the degree of predictability of the relevant art need to be considered. I have been informed that chemical and physiological inventions, unlike mechanical and electrical inventions, are often considered “unpredictable” arts because a slight variation in method can yield an unpredictable result or may not function at all. (“[I]n cases involving chemicals and chemical compounds, which differ radically in their properties it must appear in an applicant’s specification…, that the chemicals or chemical combinations included in the claims are capable of accomplishing the desired result.”) Accordingly, “[t]ypically, patent applications claiming new [chemical]

methods of treatment are supported by test results." *In re '318 Patent Infringement Litig.* 583 F.3d at 1324 (Fed.Cir. 2009). I have been informed that in unpredictable arts, there must be some quantum of data or reasoning that supports the inventor's contention that a therapy operates as claimed. See *Id.* at 1326. While every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification, reasonable detail must be provided in order to enable members of the public to understand and carry out the invention." *Genentech*, 108 F.3d at 1366.

31. I have been informed that the specification need not teach what is well known in the art. *Hybritech v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986).

32. I have been informed that the determination of non-enablement must be based on the evidence as a whole.

VI. DESCRIPTION OF THE RELEVANT FIELD AND THE RELEVANT TIMEFRAME

33. I understand that the '055 Patent issued from U.S. Patent Application 15/468,494, filed on March 24, 2017.

34. I have been instructed by counsel to assume the relevant timeframe for my analysis in this declaration to be on or before March 24, 2017.

35. Based on my review of this material, I believe that the relevant general fields for the purposes of the ’055 Patent are, as identified above, biochemistry, cell biology, genetics.

VII. OVERVIEW OF THE ’055 PATENT

A. Subject Matter Overview

36. The ’055 is directed to transgenic plant cells expressing a recombinant Aryloxyalkanoate Dioxygenase (“AAD-12”), which enzyme purportedly degrades both phenoxy auxin herbicides and pyridyloxy auxin herbicides, thus conferring resistance thereto. *See* Ex. 1001, 7:63-9:10.

37. AAD-12 is purported to catalyze reactions of the general form shown in FIG. 1 below, based on the reaction catalyzed by the homologous α-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase (“tfdA”), which has been used in transgenic plants to impart resistance to 2,4-D, a phenoxy auxin herbicide. *See id*., Fig. 1, 3:10-21, 6:46-55, 10:65-11:1.

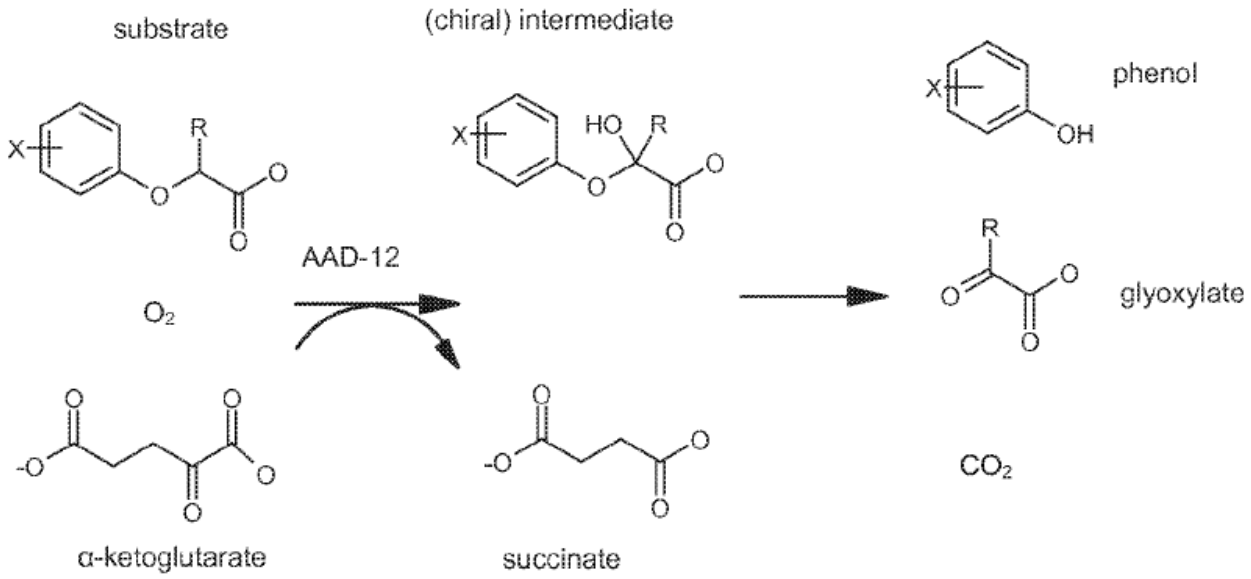

FIG. 1

38. The ’055 claims that, unlike tfdA and other homologues, AAD-12 (SEQ ID NO: 2) has the unique ability to catalyze the degradation of both phenoxy auxin herbicides (including 2,4-D) and pyridyloxy auxin herbicides. *Id*., 11:23-25. Per the ’055, AAD-12 can thus confer resistance to a wide array of herbicides in transgenic plants, allowing for cultivated plants compatible with broader and more robust weed control and herbicide resistance management options. *See id*., 4:7-16.

39. The claims of the ’055 are directed to transgenic plants comprising a recombinant polynucleotide encoding an AAD-12 protein with at least 85% sequence identity with SEQ ID NO: 2 and a conserved $HX_{109}D(X)_{111-134}H(X)_{136-261}H(X)_{263-272}R$ motif, which AAD-12 protein enzymatically degrades a phenoxy

auxin herbicide and a pyridyloxy auxin herbicide. *Id*., Cl. 1. Representative independent claim 1 of the '055 recites:

> 1. A transgenic plant cell comprising a recombinant polynucleotide that encodes an AAD-12 protein that exhibits aryloxyalkanoate dioxygenase activity wherein said activity enzymatically degrades a phenoxy auxin herbicide and a pyridyloxy auxin herbicide, further wherein said AAD-12 protein comprises:
>
> i) an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 2; and
>
> ii) an AAD-12 motif having the general formula of: $HX_{109}D(X)_{111-134}T(X)_{136-261}H(X)_{263-272}R$, wherein
>
> X_{109} represents a single amino acid at position 109, relative to the sequence of SEQ ID NO: 2;
>
> $(X)_{111-134}$ represents a sequence of 24 amino acids;
>
> $(X)_{136-261}$ represents a sequence of 126 amino acids; and
>
> $(X)_{263-272}$ represents a sequence of 10 amino acids.

Id. Independent claim 32 adds only that the conserved AAD-12 motif has 90% sequence identity with positions 108-273 of SEQ ID NO: 2. *Id*., Cl. 32. Independent claim 33 recites a method of controlling at least one weed in a field, wherein said field has been planted with transgenic seeds as recited in claim 1. *See id*., Cls. 1, 33.

B. Prosecution History

40. I have also reviewed and considered the prosecution history of the ’055 Patent, which I understand has been designated as Ex. 1002, including the sequence listings submitted 3/24/2017.

VIII. CLAIMS 1-33 LACK PROPER WRITTEN DESCRIPTION SUPPORT AND ARE NOT ENABLED

A. Claims 1-33 Lack Sufficient Written Description that Shows that Patent Owner was in Possession of the Claimed Invention

41. As discussed in §V above, I have been informed that the basic inquiry for written description is whether the specification provides sufficient information for the person or ordinary skill to recognize that the named inventors possessed the full scope of the claimed invention. It is my opinion that Challenged Claims 1-33 of the ’055 patent lack sufficient written description in the specification of the ‘055 patent (including all materials incorporated therein).

1. The AAD-12 Structure Disclosed in the ’055 Patent is Insufficient to Describe a Genus

42. Protein folding and activity is a largely unpredictable field of study, relying on trial and error as our ability to accurately predict slowly improves. The primary structure of a protein is the linear sequence of amino acids that make up the protein. Ex. 1033, pp.116-19. However, the final three-dimensional structure of a protein, which is critical for its function, is determined by a variety of factors that cannot be predicted, based on our current level of understanding, solely from the

primary sequence of amino acids. Ex. 1033, pp.120-44. The final three-dimensional structure of a protein can be determined by several factors, including the interactions between the amino acid side chains and the surrounding environment, such as the pH and temperature of the solution. Ex. 1033, pp.117-18, 148. Additionally, the folding process is often facilitated by the presence of chaperone proteins, which help guide the protein into its final shape. Ex. 1033, pp.151-153. Enzymes such as AAD-12 require a specific three-dimensional structure in order to catalyze a chemical reaction. Ex. 1033, pp.129-32. The active site of an enzyme, where the chemical reaction occurs, is typically a small region within the protein that is highly specific in terms of its shape, charge, and hydrophobicity. Ex. 1033, pp.134-41. This region is typically formed by a complex interplay of interactions between different amino acid residues that are far apart in the primary sequence. Ex. 1033, p.125. Subtle changes to the active site can impact enzyme specificity, the substrates it will recognize. Ex. 1033, pp.134-41. The variation in protein structure from such a large number of species means that this population of proteins will have widely ranging activity, from incorrectly folded proteins that have no activity or aggregate or are degraded, to those whose active site is perturbed and lack activity, to those that have their substrate specificity altered. Ex. 1033, pp.134-46.

43. Proteins have four levels of structure - primary sequence, secondary structure (e.g., alpha helices and beta sheets), tertiary structure which is the three

dimensional shape the protein formed by inter-molecular bonding such as hydrogen bonding, ionic interactions, disulphide bonds, etc., and quaternary structure formed by complexes of multiple proteins assembling together. Ex. 1016; Ex. 1028. On top of that, proteins can be regulated by permanent modifications or dynamic modifications, such as adding sugar residues, phosphate residues, etc. Ex. 1024. The active site of an enzyme, like AAD-12, is made of non-contiguous amino acids that are brought into the correct spatial configuration and are critical for catalyzing a chemical reaction. Ex. 1033, p.125. The substrates are held in a constrained geometry to allow the reaction to take place. Ex. 1033, pp.125-41. Anything that alters the shape of the active site can impact the specificity of the enzyme, the rate of the reaction and other biochemical parameters. Ex. 1033, pp.125-41.

44. The rest of the amino acids of the protein are often critical for bringing together the active-site amino acids into the correct three-dimensional configuration. Ex. 1033, pp.125-41. Amino acids can be grouped according to their chemical properties, e.g., positively charged, negatively charged, polar, and hydrophobic. Ex. 1033, pp.121-22. Substituting one amino acid that is not in the active site for another in the same group is considered "conservative" and is thought to have little impact on protein activity. Ex. 1026. This always needs to be tested because there may be an unforeseen impact on the protein's shape. Making even conservative changes to active site amino acids is likely to impact the protein's activity. As the number of

substitutions made increases, the changes to the protein structure increase exponentially, and ability to predict the impact on activity becomes more difficult, requiring direct testing of the new protein.

45. Determination of protein structure is usually done by x-ray crystallography or similar techniques. Ex. 1033, pp.136-39. The active site is usually determined by crystallizing the protein with its substrate. Ex. 1033, pp.136-39. Confirmation of active site amino acids is often done by mutating specific amino acids and looking for changes to activity.

46. There are various ways to model the structure of a new protein in the absence of an x-ray crystal structure. One way is to compare the sequence to a closely related protein of known structure. Ex. 1033, pp.141-44. Another way is to use software the predicts protein folding. Previously, protein folding software could predict well-known secondary structures, and attempt to model the 3D structure. However, the local environment where a protein is being synthesized in a cell, e.g., pH, temperature, presence of protein folding chaperones, can have a dramatic impact on how a protein folds. Ex. 1033, pp.117-18, 148.

47. The biggest advance to protein prediction software in recent years has been AlphaFold, which uses AI and deep learning to improve protein prediction, but it is still unable to predict the structure of a sizable number of proteins; this software was not available to the public until after the filing of the ’055 patent. Ex. 1021.

Even with its significant advances over prior protein prediction software, AlphaFold also cannot accurately predict all the static or dynamic protein modifications which can impact protein activity. Ex. 1022. Even knowing the structure does not guarantee the ability to accurately predict the substrates of an enzyme. Even with the advanced prediction software currently available to the field, determination of protein functionality, activity level, and substrate specificity requires the expression and purification of the target protein, and *in vitro* enzymology. With or without access to AlphaFold, a scientist seeking to engineer a protein on or around March 24, 2017 would not be able to rule out anywhere near as many sequences predicted to misfold, and would have to engage in an unduly broad campaign of protein expression, purification, and *in vitro* enzymology to optimize and/or design a protein with a target function.

48. α-Ketoglutarate-dependent dioxygenase are part of very large superfamily of enzymes which catalyze a wide range of reactions including hydroxylation, dealkylation, desaturation, epoxidation, epimerization, halogenation, cyclization, peroxide formation, and ring expansion/contraction reactions. Ex. 1017. Because α-ketoglutarate-dependent dioxygenases catalyze such a diversity of reactions, it is impossible to ascertain substrate specificity from presence of the general motif attributed to the family, of which the "AAD-12 motif" belongs to Group 2. Although the conserved amino acids of the "AAD-12 motif" that are

present in the active site are necessary for the α-ketoglutarate-dependent dioxygenase activity, notably the "AAD-12 motif" which forms the catalytic iron binding site, they are not sufficient to determine substrate specificity. An "AAD-12 motif" that is not in the correct spatial configuration, will not bind the catalytic iron.

49. Although there is flexibility in the amino acids between the histidine residues in the motif, there is still an ultimate three-dimensional arrangement which may not be predicted by a given primary amino acid sequence. Ex. 1033, pp.120-44. Additional amino acids in the active site pocket determine the shape of the substrate that can enter and bind. Ex. 1015; Ex. 1023. For example, Arg104, Asp108, Asp109, Val170, Phe182, and Val220 are critical in forming a tunnel that allows the ring structure of the auxin herbicides to be positioned in the active site. Ex. 1015. The presence of the iron binding site alone would not allow a POSITA to predict whether an α-ketoglutarate-dependent dioxygenase would interact with or degrade either phenoxy auxin herbicides and/or pyridyloxy auxin herbicides.

50. The substrate specificity of an α-ketoglutarate-dependent dioxygenase under investigation, such as AAD-12 and variants thereof, could only be accurately determined by *in vitro* testing. Thus, presence of the "AAD-12 motif" alone is insufficient to predict whether a protein of 85% or higher identity would be an α-ketoglutarate-dependent dioxygenase at all, let alone whether it could degrade both phenoxy and pyridyloxy auxin herbicides.

2. The Examples Disclosed in the Patent are not Reasonable to Support the Broad Genus

51. The Patent Owner claims that the AAD-12 protein is able to degrade both phenoxy and pyridyloxy auxins. Although the *in vitro* data demonstrates this, it is also critical to demonstrate the activity in each plant species that needs to be tolerant to auxin herbicides.

52. For each transgenic plant species, the different types of auxin herbicides must be tested before tolerance can be ascertained. The tolerance is often determined by the enzyme activity of the tolerance protein in a specific transgenic plant. Because protein expression levels and protein folding can occur differently in different species for a given novel protein, tolerance in one plant species is not predictive of tolerance in a second plant species. In fact, in Patent Owner's inventor declaration during prosecution of the patent, the inventor noted "there is a high level of unpredictability associated with expressing bacterial genes in plant systems", highlighting that AAD-2, despite having significantly higher activity with 2,4-D *in vitro* than AAD-1 (both tfdA homologs), "was surprisingly inactive when expressed in plants, while AAD-1 was very active." Ex. 1010, ¶¶9-10.

53. In this case, Patent Owners did not perform all experiments with each species of transgenic plant. Although tolerance was tested in transgenic tobacco, canola, corn and Arabidopsis for 2,4-D, tricyclopyr and fluroxypyr, rice was tested only for tolerance to 2,4-D and tricyclopyr, transgenic soybeans were only tested for

tolerance to 2,4-D, and no data was disclosed for transgenic cotton tolerance to any auxin herbicide. There is no data to show that transgenic soybeans have pyridyloxy auxin tolerance, and there is no data to show that cotton plants have tolerance to either auxin herbicide class. Yet, for example, claim 3 of the '055 patent specifically recites soybean and cotton transgenic plant cells. As noted above, there is no support for such explained anywhere in the specification of the '055 patent.

54. In addition to the gaps in the testing in the '055 patent, the Patent Owner's scientific paper on the subject, also fails to demonstrate pyridyloxy auxin herbicide tolerance in soybean, and no data is shown on cotton. Ex. 1005. Although in their own publication the Patent Owner indicates that both 2,4-D and "-fop" (that is some pyridyloxy) herbicides can be used on Enlist Corn, for Enlist E3 Soybeans only 2,4-D can be used. Ex. 1029, *also* Ex. 1006, Ex. 1011, Ex. 1012. That is consistent with the lack of data in their patent and PNAS publication, but also contradicts the patent claims of dual auxin class tolerance.

55. As discussed above, just because a transgene cassette functions in one plant species, does not mean that it will function equally in another plant species. It is critical to test the transgene in each plant species that will be used. In the case of AAD-12, it is also important to test the full spectrum of auxin herbicides, both phenoxy and pyridyloxy, to demonstrate that the activity is present. The Patent Owner here failed to do so by demonstrating the presence of the ability to degrade

both phenoxy and pyridyloxy auxin herbicides in only a subset of the AAD-12 transgenic plants expressly disclosed.

B. Significantly More Support Would be Necessary for the Broad Genus Claimed

56. As discussed in §V above, I have been informed that a set of "Wands" factors several outline aspects to analyze for a determination as to whether a claimed invention is enabled for use by others of ordinary skill in the art without undue experimentation, and that these factors include "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims." *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). I address these Wands factors below. It is my opinion that Challenged Claims 1-33 of the '055 patent are invalid because they require degrading phenoxy auxin herbicides and pyridyloxy auxin herbicides at 85% sequence identity with SEQ ID NO: 2, which is not enabled, as explained below.

57. In my opinion, analysis of Wands factor (1) the quantity of experimentation necessary shows that practicing the invention would require substantial experimental work because any amino acid sequence differing from the AAD-12 species disclosed would have to be subjected to a complete biochemical analysis *in vitro* and *in vivo* to determine whether it had the claimed enzymatic

activity without any hint as to what structural features are key to that activity, as discussed in paragraphs 86-93 below.

58. In my opinion, analysis of Wands factor (2) the amount of direction or guidance presented shows that practicing the invention would require undue experimentation because the direction or guidance provided relates only to a single species, which itself showed activity in only some transgenic plants, and does not disclose structural features responsible for imparting the desired enzyme activity to proteins, as discussed in paragraphs 79-85 below.

59. In my opinion, analysis of Wands factor (3) the presence or absence of working examples shows that practicing the invention would require undue experimentation because only a single working example was provided, and even that single working example only demonstrated the claimed functionality in some, but not all, of the transgenic plants disclosed, as discussed in paragraphs 80-82 below.

60. In my opinion, analysis of Wands factor (4) the nature of the invention shows that practicing the invention would require undue experimentation because it protein engineering and genetic modification is a complicated field involving a vast array of variables which must be considered, assayed, and accounted for, as discussed in paragraph 78 below.

61. In my opinion, analysis of Wands factor (5) the state of the prior art shows that the prior art primarily deals with enzymes of the same general family that

nonetheless lack the claimed functionality, and specifically includes data only as to the native AAD-12, as discussed in paragraphs 65-70 below.

62. In my opinion, analysis of Wands factor (6) the relative skill of those in the art shows that a very high level of skill in the biochemical arts is required for the genetic modification of plants, as discussed in paragraphs 71-75 below.

63. In my opinion, analysis of Wands factor (7) the predictability or unpredictability of the art shows that practicing the invention would require undue experimentation because the ability to predict protein folding, which was at the time generally unreliable, would not inform as to enzymatic activity, active site structure, or substrate specificity, and thus detailed biochemical analysis would be required for any amino acid sequence of interest, as discussed in paragraphs 71-77, 80, below.

64. In my opinion, analysis of Wands factor (8) the breadth of the claims shows that practicing the invention would require undue experimentation because the claims cover a universe of 2.4×10^{106} potential species, as discussed in paragraph 79 below.

1. Extensive and Undue Experimentation is Required to Practice the Full Scope of the Claimed Invention

65. Glyphosate tolerant soybeans were released in 1996; glyphosate tolerant corn was released in 1998. Ex. 1034, p.332. These two products had a very large impact on farming practices and herbicide usage. Ex. 1034, p.332. No longer reliant on older selective herbicides, farmers could use primarily glyphosate, an

inexpensive broad-spectrum herbicide that could be used for both pre-planting burn downs, as well as early and late season weed control. Ex. 1034, pp.330-34. However, this reliance on a single herbicide on greater than 90% of corn and soy acres, and because most corn farmers rotate their corn crop with soy led to herbicide resistant weeds. Ex. 1034, pp.330, 333.

66. Glyphosate-resistant weeds were first observed in 2000 in the US. By 2018, there were glyphosate-resistant weeds in the majority of soybean-producing States. Ex. 1034, pp.332-33. Because these weeds were not only resistant to glyphosate, but like palmer amaranth, can produce upwards of 1 million seeds per plant, farmers needed alternative herbicides. Ex. 1034, pp.330-34. The need to develop alternative herbicide-tolerance systems in crop plants stimulated investigation of alternative herbicide tolerant modes of action using already approved herbicides. Ex. 1034, pp.330-34.

67. The auxin class of herbicides represents a very diverse set of herbicides. 2,4-D, a synthetic auxin, was first used in the 1940s. Ex. 1034, p.330. Auxin herbicides work by overstimulating plant cells (auxin is a growth promoting plant hormone) and causing plant death. Ex. 1034, pp.330-34. Work on auxin-degrading enzymes often started with isolating microorganisms from contaminated soil which degraded auxin. Ex. 1034, pp.330-34.

68. The first auxin-degrading enzyme, tfdA, was identified in 1987. Ex. 1025. TfdA is in the α-ketoglutarate dioxygenase superfamily of enzymes that catalyze a wide range of reactions including hydroxylation, dealkylation, desaturation, epoxidation, epimerization, halogenation, cyclization, peroxide formation, and ring expansion/contraction reactions. Ex. 1017. While TfdA expressed in transgenic plants can give tolerance to 2,4-D, there was a negative impact on yield.

69. Groups working on other phenoxy auxin degrading microbes identified additional proteins that were part of the same α-ketoglutarate dioxygenase family, but they had differing selectivity for the different auxin herbicides. Ex. 1023; Ex. 1027. One of these was AAD-12. The Patent Owner used AAD-12 to generate auxin herbicide-tolerant plants. Ex. 1001; Ex. 1005. Another class of auxins includes the herbicide Dicamba, which is a benzoic acid auxin. Dicamba monooxygenase (DMO) was identified from a soil bacterium. Ex. 1014. The enzyme that breaks down dicamba does not degrade phenoxycarboxylic acid auxins. DMO was engineered into soybean and corn to give another mode of herbicide tolerance. Ex. 1034, p.333. Dicamba tolerance was released by Monsanto in 2015 for cotton and in 2016 as part of its Roundup Ready® Xtend System in soybeans.

70. During the investigations of enzymes that degrade synthetic auxin herbicides, one enzyme had particular interest due to the specificity of its activity.

Originally called sdpA and later renamed AAD-12 by the Patent Owner, this enzyme was very efficient in its degradation of the phenoxy auxin herbicide 2,4-D. Ex. 1027. It was then found to have significant activity on the pyridyloxy auxin herbicides triclopyr and fluroxypyr. Ex. 1005. The ability to degrade two discrete classes of synthetic auxin herbicides made AAD-12 a desirable enzyme to use to engineer auxin-tolerant crops. It would give farmers greater flexibility in the use of synthetic auxin herbicide on their crops.

71. The field of engineering enzymes for herbicide tolerance in plants is quite complex, and it requires specialist knowledge and experience in several areas for selecting the proper enzyme, generating the transgenic plant, and evaluating the herbicide tolerance. One needs to understand the action of the herbicide in planta and how it is lethal in order to choose a strategy of tolerance. Choosing an enzyme, the scientist needs to understand detailed biochemistry to be able to test *in vitro* whether enzymes can degrade the herbicide effectively. If the enzyme needs to be modified, the scientist requires protein chemistry, genomics and molecular biology skills to either search in databases for homologous proteins, or to design and introduce mutations to the existing protein.

72. Once a protein is chosen, the scientist needs to know how to choose the appropriate gene expression cassette, including promoter, terminator, other expression regulatory sequences, as well as how to codon optimize the coding

portion of the gene for a given plant species. This expression cassette likely requires modification for each plant species that will be transformed.

73. The experimenter further needs to be skilled in plant transformation. Although there are published protocols, it often requires a high degree of skill to efficiently generate transgenic plants. Plant transformation is not completely predictable. The location a transgene inserts in the plant genome can have a large impact on expression of the heterologous protein. Also, transgenic plants often have multiple copies of the expression cassette. For this reason, a large number of independent transgenic plants need to be generated for each transgene being evaluated.

74. Although protein expression cassettes can be designed to optimize expression of a heterologous protein in plants, meticulous experimentation is required to confirm the proper expression of the new protein. There are numerous processes that can impact successful expression of the protein. These include where the gene expression cassette inserts into the plant's chromosome, how many copies of the gene expression cassette are incorporated into the plant's chromosome, whether gene silencing has been triggered, the proper folding and accumulation of the protein, etc. Herbicide tolerance from a herbicide-degradative protein, like AAD-12, requires a proper level of expression. Because there is a lot of variability of transgenic protein expression between independently transformed plants, many need

to be generated to properly evaluate any single protein variant. If the enzyme catalyzes a reaction with any native substrates, it could also impact the growth and development of the plant. Sometimes off-target enzyme activities are not able to be predicted but only observed once a transgenic plant is generated.

75. Once the transgenic plants are grown in the greenhouse or growth chamber, the scientist needs to be skilled in protein and nucleic acid analytic techniques to confirm the presence of the transgene, number of copies, intactness of the inserted DNA, its location in the genome, and the expression of the protein. The plants need to be propagated for several generations to produce homozygous seed for testing of the herbicide tolerance in planta. For evaluating herbicide tolerance, the scientist needs to understand how to apply the herbicide, the rates, the timing, and how to evaluate the plants for damage.

76. Since the beginning of research on transgenic plants, variation of protein expression between independently transformed plants with the same gene expression cassette has been observed. Often it is termed the 'position effect' because the location of the transgene in the plant's chromosome, the 'position', is one of the causes of the variation. The common method of plant transformation using *Agrobacterium tumefaciens* results in random insertion of the transgene in the chromosome. In Ex. 1018, the authors compared the expression of two different reporter genes, an antibiotic resistance gene chloramphenicol acetyltransferase

(CAT) and a color-producing enzyme 3-glucuronidase (GUS). Both genes were on the same expression cassette. The authors did not evaluate expression when one of the proteins was zero, indicating that variation was even higher than the data at first indicates. Looking at 45 lines that expressed both proteins, they found that expression of the proteins varied between 136- and 175-fold among lines. Even more telling, the ratio of expression of the two proteins varied between 0.05 and 49. Because the degree of protein expression is linked to the performance of an enzyme, a number of independent transgenic events need to be generated to properly assess a novel protein's expression and performance in plants.

77. In the '055 patent, the Patent Owner demonstrates some of the variability that they found in protein expression between transgenic lines. For example, in the '055 patent, they measured protein levels of AAD-12 in soybean as shown in Table 25 (75-78). Using the AtUbi10 promoter, the level of protein ranged from 4.65 ng/ml to 2657.36 ng/ml among 7 events they reported. That is an over 570-fold range of expression. Looking at their AAD-12 expression in canola, Table 29 (96-97), among 5 samples they find the protein levels range from 41.36 ng/ml to 3879.09 ng/ml, a 94-fold range of expression. Depending on protein level, you can get differing amounts of herbicide tolerance. This is demonstrated in Table 20 of the '055 patent (68-69), where their evaluation of herbicide tolerance in tobacco shows variation from tube to tube. As their own data shows that a wide range of expression

and herbicide tolerance is inherent in the plant transformation process. Therefore, as part of the evaluation of any single protein or protein variant, a number of independently transformed plants need to be evaluated before it is known how well that protein performs in plants.

78. The invention disclosed in the '055 patent includes five different crops (cotton, tobacco, canola, soybean and Arabidopsis), that are expressing a transgenic protein that is at least 85% identical to AAD-12 and contains the "AAD-12 motif". These crops would be tolerant of commercial levels of the synthetic phenoxy auxin herbicide 2,4-D as well as commercial levels of the synthetic pyridyloxy auxin herbicides, like triclopyr and fluroxypyr. This auxin herbicide tolerant trait could also be able to used in combination with other herbicide tolerant traits listed in the patent (including glufosinate tolerance, glyphosate tolerance, HPPD inhibitor tolerance, PPO inhibitor tolerance, ALS inhibitor tolerance and dicamba tolerance) to give a multi-herbicide tolerant crop system for farmers to be able to control a wide spectrum of weeds.

79. The claims in the '055 patent cover all enzymes capable of degrading both phenoxy and pyridyloxy auxin herbicides that are at least 85% identical to AAD-12 and contain the conserved "AAD-12 motif". When the number of different potential proteins that fall within this specification is calculated, an extremely large number is revealed (over 10^{106} unique proteins). Although not all of these will

function as an effective synthetic auxin degradative enzyme that can be expressed well in crop plants, the Patent Owners do not provide any other criteria to determine which of these in the universe of proteins will have the necessary activity. They do not provide any testing of other variants of AAD-12 to indicate other criteria that is necessary. For example, the Patent Owner could better define the active site through a set of spatial coordinates of key amino acids and distances. This would define a much smaller, and more meaningful set of proteins. The Patent Owner could also restrict mutations to conserved amino acids, as well as predicted secondary and predicted tertiary structures. Without any of these specifications, the only way to identify AAD-12 variants that have a similar activity and function is through a series of modeling, *in vitro* and *in planta* experiments. These would take excessive amounts of resources and time.

80. As discussed below, the predictably of protein folding, protein structure and enzyme specificity is, and, as of March 24, 2017, was in its infancy. From a primary amino acid sequence, it is not possible to predict all protein folding with a high degree of accuracy, and even less so substrate specificity. The Patent Owners claim all proteins having a specific enzymatic activity with 85% identity to AAD-12, and the only required amino acids being 5 in the "AAD-12" motif. This is an

extremely large number of proteins (more than 10^{106}).[1] They have only presented characterization of a single species of protein. They also have not revealed any other active site amino acids that are known to be necessary for phenoxy or pyridyloxy auxin herbicide degrading activity. The 85% sequence identity constraint is an arbitrary number lacking any experimental support.

81. They have not tested any other variants to be able to say what is special about 85% and higher homology. Nor do they show that less than 85% homology makes a protein any less of an auxin herbicide degrader. The Patent Owner did not demonstrate pyridyloxy auxin herbicide tolerance in soybean, nor phenoxy or pyridyloxy auxin herbicide tolerance in cotton. Demonstrating enzyme function *in vivo* is a critical step, because not all enzymes work equally well in all plant species, as shown by the Patent Owner and described in detail below.

82. Besides the AAD-12 sequence, the Patent Owners do not give any indication to what sequences in the very large space of 85% identity (2.4 x 10^{106}

[1] To calculate the number of different amino acid combinations possible, I used the equation C = n!/((n-r)!*r!), where n is the number of amino acids that can be changed, and r is the maximum number of changes. For AAD-12, which has a length of 292, 5 invariant amino acids in the "AAD-12 motif" and the Met start codon, n = 286. Because the protein needs to be at least 85% identical to AAD-12, r = 43. Because the number of amino acid substitutions for any single site is 19, the number of variants with one substitution is 19^{43}. So the total number of variants is determined by the equation C = 19^{43}*(286!/((286-43)!*43!), which equals 2.4 x 10^{106}. For 90% identity as claimed in claim 2, the number of variants is on the order of 10^{76}. For 85-90% identity as claimed in claim 32, the number of variants is on the order of 10^{86}.

different proteins fit this description) would be able to degrade both phenoxy and pyridyloxy auxin herbicides. Certainly, many of these proteins will be worse than AAD-12 because the substitutions alter the 3-dimensional shape too much and eliminate, obstruct, or otherwise degrade the active site. However, there are probably many that, due to the mutations being conservative in nature and thus not substantially altering the shape of the active site, would work at a level equivalent to the disclosed AAD-12. There may even be some substitutions that result in a better enzyme. Absent extensive and undue experimentation, as described above, it would be impossible to determine the effects of the vast majority of mutations on the function of AAD-12.

83. A specific enzyme's amino acid sequence will lead to a properly folded protein with the critical active site amino acids precisely positioned to carry out its chemical reactions. The Patent Owners only include part of the active site, the catalytic iron binding site, but not active site amino acids required for binding the auxin herbicide substrates, in the structure disclosed for the genus. Substitutions in the rest of the amino acids may be conservative – meaning they have minimal effect, however, even subtle changes in amino acid size, charge, and/or polarity can have big impacts on enzyme activity. Some of these are predictable; many are not.

84. The Patent Owners also do not give guidance on what type of conservative amino acid substitutions might be permissible that would retain the

spatial arrangement of the active site. Without this guidance, the only way to determine whether proteins that contain the "AAD-12 motif" and are at least 85% identical to AAD-12 have an appropriate and functional active site spatial arrangement would be to carry out the entire inventive process of modeling, cloning, transformation and expression, enzymology and *in vivo* testing anew, in order to determine which proteins might have the desired auxin herbicide degradative activity.

85. The Patent Owners additionally do not give any guidance as to which types of mutations fall into the different categories (Better enzyme, equivalent enzyme, worse enzyme). The only way to determine which category a variant falls into is through the combination of modeling, *in vitro* testing and *in vivo* testing described above. The numbers of variables involved in these processes are substantial, and accounting for and optimizing the system to account for such variables would be extreme and undue.

86. Analyzing the activity of AAD-12 variants is a complex and time-consuming undertaking, with no guarantee of success given the sheer number of variables involved in protein folding and substrate specificity and lack of guidance from the '055 specification as to what structural features are necessary to impart the claimed activity of enzymatically degrading both phenoxy and pyridyloxy auxin herbicides. The number of discrete proteins with at least 85% identity to AAD-12

and having the "AAD-12 motif" is over 10^{106}. An experimenter could only test a tiny fraction of these, with no guarantee of success. Nevertheless, the testing required would be overwhelmingly based in trial-and-error, as any individual amino acid sequence would require a complete and detailed biochemical analysis and, in effect, constitute a new invention unto itself; the only substantive guidance provided is the desired function and single species capable, in at least some circumstances, of that function.

87. Computational resources for protein prediction available as of March 24, 2017 might, in some instances, indicate which proteins would clearly misfold. This could be used as a criterium for deciding which proteins to work on *in vitro*. But because protein folding software gives a 3-dimensional output, not a yes/no answer, there would be no indication or guidance as to whether an amino acid sequence with an acceptable predicted tertiary structure would have any functionality at all, much less the ability to degrade both phenoxy and pyridyloxy auxin herbicides. There would further be no guidance as to changes to make to "fix" the protein or impart the desired functionality to it or improve it. Given the lack of predictability for protein design, engaging in such would ultimately be fruitless; the only option would be blind trial and error of amino acid sequences falling within the claim in order to invent another species capable of the claimed function.

88. Even with AI enabled software of today, there is inadequate resolution necessary to determine the precise configuration of the active site, which is necessary to predict activity A current-day experimenter might be able to predict gross changes in protein structure, but could not differentiate the many proteins that would have smaller changes in structure that would impact enzyme activity – even with the more advanced analysis tools available today.

89. As such, *in vitro* testing is necessary to verify the enzyme activity. The nucleotide sequence encoding the amino acid sequence predicted to fold in an appropriate manner would need to be synthesized, transformed into a proper protein expression system, like *E. coli*, and then the protein isolated and tested for auxin herbicide degradation activity. Proteins can be poorly expressed in different host organisms for a wide variety of reasons, and for each target protein expression at a useful level and in a soluble structure would have to be assessed.

90. Although in the ’055 patent, there is some specification for the characteristics of the protein that would be at least 85% identical to AAD-12 and function as an auxin herbicide degradative protein, there is insufficient guidance to be able to predict the activity from this level of specification. The key feature that is included is the “AAD-12 motif”. There is no further description of amino acids that are required for binding either phenoxy or pyridyloxy substrates which could be used in identifying other active enzymes that are 85% or more identical to AAD-12. As

described in Ex. 1019, where the authors examined the active site of TfdA, they found the consensus sequence H*X*(D/E)*X* $_{138–207}$H*X* $_{10}$R/K was critical for binding the catalytic iron residue and potentially one histidine, H213, that may be involved in either catalysis or 2,4-D binding. Similarly, in Ex. 1023 the authors report on the motif $HXDX_{24}TX_{131}HX_{10}R$ which is required for iron binding. Moreover, they commented that a large number of residues participate in the directing the specificity of the substrates. These residues were not identified in this paper. In Ex. 1020, the authors further investigated the active-site residues that might be responsible for the differing enantiomeric specificity between RdpA and SdpA (AAD-12) for the chiral phenoxy auxin herbicides mecocop through a combination of protein modeling and site-directed mutagenesis. Notably SdpA does not degrade mecocop. They found several residues were likely responsible for mecocop binding. However, they did not identify them as critical for other auxin herbicides. There has been a more recent publication, Ex. 1015, which identified critical active site amino acids.

91. Although *in vitro* data is an important first step in trait testing, it does not guarantee performance in planta. There are a number of biological processes that could prevent an enzyme from performing as needed. Detailed experimental analysis is required to determine how well an herbicide tolerant trait is functioning in planta, and to isolate and modify the variable(s) responsible so as to obtain the desired herbicide resistance in a single species of plant. The plant expression vector

may not produce enough protein. Or if it produces too much protein, it could trigger a process known as transcriptional gene silencing that reduces the expression of transgenes. Due to the local environment of the plant cell (e.g., temperature, pH, presence of protein folding chaperone proteins, etc.), the protein may not fold correctly in the plant cell resulting in proteolytic degradation and a resulting lack of efficacy. The protein may also be recognized by protein modification enzymes that trigger addition of phosphate groups or sugar groups that impact enzyme activity. In each case, the experimenter would need to design and optimize experiments to identify the factors impacting the trait and determine how to improve the system to achieve the desired effect.

92. Those enzymes that demonstrate the desired activity *in vitro* would thus have to be tested *in vivo*, as no guidance is provided as to how *in vitro* results correlate to *in vivo* efficacy beyond the single species tested. A new set of plant gene expression constructs would need to be made, as protein expression constructs designed for microorganisms are not generally functional in plants. Plant transformation typically takes 6 weeks to 8 months (depending on the plant species). Each species of plant, requires a specific protocol for transformation, including the production of target tissue, which may be a leaf piece, an embryo, a callus, etc. This increases the effort and challenge to generate multiple species of transgenic plants. Ex. 1013. Multiple transgenic plants for each enzyme variant would need to be made.

The insertion site for a transgene can have a large impact on not just expression of the target gene, but it can cause disruption of native genes which would impact the growth and development of the plant. For example, if the transgene disrupts or knocks out the expression of a native this can be lethal in the extreme, or just cause an altered growth habit. Expression analysis would be required to determine which plants are producing the enzyme in sufficient quantities, which itself would vary by plant species. Here, too, the '055 patent provides only effective expression levels of a single AAD-12 enzyme in 5 different species of plant; this data provides no guidance as to effective expression levels of other amino acid sequences for a measurable *in vivo* effect. Then, the selected transgenic plant strain(s) could be used to test the performance of the enzyme in planta. Plant transformation and growing plants require a lot of resources, and would limit the number of enzyme variants that can be practically tested.

93. Finally, even if the protein is present and sufficiently expressed in the transgenic plant, it must be tested for tolerance to the herbicide sprayed at commercial levels. Herbicide resistance is accurately measured starting in T2 (second generation) of transgenic plants. The plants would need to be grown until 3 or 4 sets of leaves are present, and then treated with the target herbicide at commercial rates. After 2-3 weeks the plants are scored for % damage. The testing of herbicide traits is quite intensive. Typically, for robust herbicide tolerance testing,

the plants need to be tested for germination in the presence of the herbicide, as well as sprayed at different times during the growth season. Weeds may be present before planting, or they may start growing anytime up to harvest. It is important to see that the plant is tolerant to the herbicide throughout the lifecycle. Also, the plant is tested to ensure it can survive multiple treatments, and at levels 2-6 times greater than the commercially recommended treatment for weeds. In addition, environmental conditions (soil type, soil moisture, weather, temperature, and other environmental conditions), may have an impact on the performance and/or expression of the protein. Therefore, the plants need to be tested in multiple growing environments. For all of these, crop damage at different time points, looking at initial damage, if any, and recovery, and finally crop yield needs to be assessed. So, the number of protein variants that it is feasible to test is quite low. And in the event that the desired herbicide resistance is not seen at commercially-relevant levels, the entire process would need to be reviewed, analyzed, and assessed for potential steps to take to achieve the desired result.

IX. CONCLUSION

94. In summary, I have concluded that claims 1-33 of the ’055 Patent fail to comply with written description and enablement requirements, and therefore claims 1-33 are unpatentable under 35 U.S.C. §112.

95. I currently hold the opinions expressed in this declaration. But my analysis may continue, and I reserve the right to supplement my expert opinions in the future to respond to any arguments that Patent Owner or its expert(s) may raise and to take into account new information as it becomes available to me.

96. In signing this declaration, I recognize that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I also recognize that I may be subject to cross-examination in the case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

97. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: 03/28/23 By: _____

Dr. Aron Silverstone
Durham, NC

APPENDIX A

(C*urriculum Vitae*)

ARON L. SILVERSTONE, PHD, PMP

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Durham, NC 27705

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Skills and Expertise

R&D leader experienced in biotechnology and crop protection product development. Diverse scientific and project management background including collaborating with internal and external stakeholders, leading team members, directing projects, and driving strategic planning.

- Project Management
- Collaborations
- Communication
- Team Leadership
- Change Management
- Risk Management
- Consensus Building
- Report Writing
- Experimental Design
- Data Analysis
- Molecular Genetics
- Plant Physiology

Professional Experience

AgStone Research, LLC – Durham, NC
Consultant, 02/2023 - present

- Biotechnology
- Agriculture

Syngenta Crop Protection, LLC – Greensboro, NC

Technical Leader Residue Science, Human Safety 11/2020 – 02/2022

- Led team of scientists overseeing residue studies for regulatory submissions in US and Canada.
- Supported the product development of new active ingredients and expand the use of registered pesticides.
- Interacted with regulatory and marketing colleagues to identify opportunities for robust product performance.
- Improved efficiency of internal processes.
- Served on leadership teams to ensure Residue Science perspective contributes to strategic decisions.

North America Lead Product Safety Operations 1/2019 – 10/2020

- Led team of specialists supporting 100+ Product Safety scientists for budgeting, outsourcing, document management, knowledge management and lab management
- Ensured alignment with global Product Safety practices.
- Developed projects to improve efficiency of the Product Safety group.

North America Product Biology Lead, Abiotic Stress Management, 6/2014 – 12/2018

- Oversaw field trial program to test products for performance in mitigating yield loss from abiotic stresses.
- Designed trials and testing methods to identify crop enhancement benefits from fungicides.
- Worked with field scientists on protocol design and data analysis.
- Ensured product availability for field and greenhouse testing.
- Updated planning tools.
- Interfaced with product marketing and assets teams.

Syngenta Seeds – RTP, NC

Global Project Manager, Soybean Traits 7/2010 – 5/2014
Herbicide-Tolerant Soybean

- Executed development project for a next generation HPPD-tolerant soybean.
- Managed multifunctional project team including field biology, regulatory, product safety, marketing, IP and legal.
- Coordinated between Seeds and Crop Protection projects.
- Oversaw co-development project with Bayer Crop Science.

Insect Control Soybean

- Directed development of a novel insect control trait stack for the LATAM market.
- Managed multifunctional project team.
- Activities included coordinating lab studies, field activities, IP, product safety and regulatory, and marketing.
- Led effort to develop a software tool that simulates Event Selection and Trait Introgression for soybean for use in scenario testing and decision analysis.

Syngenta Biotechnology, Inc. – RTP, NC

Senior Project Leader 4/2007 – 6/2010

- Developed technologies to enhance our abilities to express heterologous proteins in plants.
- Worked with internal research team, 3rd party collaborators and licensing opportunities.

Project Leader 6/2003 – 3/2007
Raw Starch Project

- Led research team and was project manager to develop next generation starch to ethanol technology.
- Managed effort to discover and validate novel enzymes with external collaborator.
- Generated and analyzed transgenic plants expressing novel starch hydrolytic enzymes.
- Managed coordination with Trait Introgression, Product Safety, Regulatory and IP teams.

Golden Rice Project

- Developed technical strategy to generate, characterize, and select lead events for an improved Golden Rice (high beta-carotene rice).
- Identified events with up to 20-fold higher beta-carotene than original Golden Rice.

- Golden Rice lead events were donated to the Humanitarian Board for oversight and product development.

Enogen Project

- Led research team supporting the corn starch to ethanol project.
- Designed and analyzed lab experiments, fermentation trials, ethanol pilot plant trials, and field trials.
- Collaborated with 3rd party ethanol producers

Staff Scientist II, 2/2001 – 5/2003

- Discovered and validated seed-specific promoters.
- Worked with external collaborator to enhance soybean for animal feed.

Staff Scientist I, 6/1999 – 1/2001

- Designed and built constructs to overexpress sweet protein in corn.
- Analyzed transgenic events.

Developmental, Cell and Molecular Biology Group, Department of Botany, Duke University
Postdoctoral Fellow, 4/1994 – 5/1999

- Conducted research on the biosynthesis and signal response pathway of the plant hormone gibberellin in the model plant Arabidopsis.
- Discovered that key steps in GA signaling involved the degradation of repressor proteins.
- Identified cellular locations of early steps of GA biosynthesis throughout plant development.

Department of Vegetable Crops, University of California, Davis
Research Associate, 8/1987 – 3/1994

- Performed research on the biosynthesis of the plant hormones ethylene and auxin.
- Identified novel metabolism reactions.

Education and Professional Training

University of California, Davis, Ph.D., Plant Physiology 1994
Harvard College, A.B., *magna cum laude*, Biology 1987

Leadership Effectiveness Program UNC Kenan-Flagler Business School 2021
Foundations of Advocacy Training Syngenta Product Safety, 2020
GLP Training Syngenta Quality Assurance Unit, 2020
Manager of Managers Pathway Program, Kincentric, 2019-2020
MSP (Managing Successful Programmes, Registered Practitioner), 2012
Project Management Professional (PMP) Certification, Project Management Institute 2009
ALPS (Agribusiness Leadership Plus at Purdue), 2003-2004
Managing Innovation in Agribusiness Program, UNC-Chapel Hill, 2001

Invited Talks

RTP 180, Research Triangle Park, NC (2019)
Shanxi Normal University, Linfen, China (2018)
International Plant Growth Substances Association Meeting, Toronto, Canada (2016)
Annual Meeting of the Plant Pathology Society of North Carolina, RTP, NC (2010)
Frontiers in Transgenesis, Donald Danforth Plant Science Center, Creve Coeur, MO (2007)
Crop Improvement for Human Health, MSU (2005)
North Carolina Plant Molecular Biology Consortium, 14th Annual Retreat (2000)
ASPB Annual Meeting, Baltimore, MD (1999)
Plant Gene Expression Center, USDA, Albany, CA (1999)
University of North Carolina, Chapel Hill (1999)
University of California, Riverside (1999)
RIKEN (Saitama, Japan) Frontiers of Gibberellin Research, Part V (1998)
Wake Forest University (1998)
North Carolina Plant Molecular Biology Consortium, 11th Annual Retreat (1997)
RIKEN (Saitama, Japan) Frontiers of Gibberellin Research, Part III (1995)
Duke University: Developmental, Cell and Molecular Biology Group (1994)

Honors

Rising Sun Award, 16th International Conference on Plant Growth Substances, 1998
Jastro-Shields Research Fellowship, 1991-1992
McKnight Foundation Fellow, 1987-1989

Teaching Experience

Biotechnology Seminar class (Bio 280S) guest lectures, Fall 2002 – present, Duke
Graduate Careers Class, panel discussion, 2014 to 2021, Duke University
Graduate Class Bio Bootcamp, panel discussion, 2017 to 2021, Duke University
Biotechnology Seminar class (Bio 195S.01) guest lectures, Fall 2001 - 2017, Duke University
Biotechnology Seminar class (Bio 199) guest lectures, Fall 1999, 2000, Duke
Developmental Biology lab class (Bio 185), guest lectures, Fall 1997, Duke
Molecular Basis for Plant Growth and Development, guest lectures, Spring 1997, Duke
Supervised Independent Study Undergraduates (16), Summer 1994-1999, Duke
Introductory Biology 1C Lab. (Plants, Fungi, Algae), Spring 1992, Spring 1993
Supervised Independent Study Undergraduate (1), Fall 1991-Spring 1992

Publications

Azhakanandam K, **Silverstone A,** Daniell H, Davey MR, eds. (2015) *Recent Advancements in Gene Expression and Enabling Technologies in Crop Plants*. Springer.

Silverstone AL, Tseng T-S, Swain SM, Dill A, Joeng SY, Olszewski NE and Sun T-p (2007) Functional analysis of SPINDLY in gibberellin signaling in Arabidopsis. *Plant Physiol*. 143:987-1000.

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Addams JL, **Silverstone AL** and Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat. Biotech*. 23: 482-487.

Silverstone AL, Jung H-S, Dill A, Kawaide H, Kamiya Y, and Sun, T-p (2001) Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *Plant Cell* 13: 1555-1565.

Sun T-p and **Silverstone AL** (2000) Gibberellins and the green revolution. *Trends Plant Sci* 5:1-2.

Silverstone AL, Ciampaglio CN, and Sun, T-p (1998). The Arabidopsis *RGA* locus encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* 10:155-169.

Silverstone AL, Mak PYA, Casamitjana Martínez E, and Sun T-p (1997). The new *RGA* locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*. *Genetics* 146: 1087-1099.

Silverstone AL, Chang C-W, Krol E, and Sun T-p (1997). Developmental regulation of the gibberellin biosynthetic gene *GA1* in *Arabidopsis thaliana*. *Plant J*. 12:9-19.

Wu YM, **Silverstone AL**, Liu Y, and Yang SF (1995) Partial purification and characterization of tryptophan malonyltransferase from tomato leaves. *Phytochem*. 40:699-704.

Liu Y, **Silverstone AL**, Wu YM, and Yang SF (1995) Formation of *N*-malonyl-L-tryptophan in water stressed tomato leaves. *Phytochem*. 40:691-697.

Dong JG, Olson D, **Silverstone AL**, and Yang SF (1992) Sequence of a cDNA coding for a 1-aminocyclopropane-1-carboxylate oxidase homolog from apple fruit. *Plant Physiol*. 98:1530-1531.

Kim WT, **Silverstone AL**, Yip WK, Dong JG, and Yang SF (1992) Induction of 1-aminocyclopropane-1-carboxylate synthase mRNA by auxin in cultured apple shoots and mungbean hypocotyls. *Plant Physiol*. 98:465-471.

Einset JW and **Silverstone AL** (1987) Hydroxylation of N^6-(Δ^2-isopentenyl)adenine to zeatin. *Plant Physiol*. 84:208-209.

Patents

US Patent 9,125,357 (2015). Methods for the commercial production of transgenic plants. Kenneth A. Dallmier, Rene Quadt**, Aron Silverstone**

US Patent 8,343,747 (2013)**.** *Amylases and glucoamylases,* nucleic acids encoding them and methods for making and using them. Ellen Burke, Michelle H. Cayouette, Cathy Chang, Majid Ghassemian, Uvini Gunavardena, Geoff Hazlewood, Peter Luginbuhl, **Aron Silverstone**, Malgorzata Slupska, Yan Zhang

US Patent 8,124,841 (2012). Truncation of the C-terminal end of alpha-amylase. Andrew DeBrecht, **Aron Silverstone**, Yan Zhang, Shengsheng Zhang, Xuejun Zhong

US Patent 7,914,993 (2011). Process for starch liquefaction and fermentation. Christopher J. Batie, Greg Crabb, George W. Aux, Edward S. Cates, Jay Austin Dinwiddie, **Aron Silverstone**, Rene Quadt, Carl A. Miller

APPENDIX B

(Documents Considered)

Exhibit ("Ex.")	Description
1001	U.S. Patent No. 11,371,055 ("'055")
1002	File History of U.S. Patent No. 11,371,055
1004	Corteva Agriscience – Technology Use Agreement (8/2022) (https://www.corteva.us/content/dam/dpagco/corteva/na/us/en/files/trait-stewardship/DOC-Corteva-Technology-Use-Agreement-WATERMARK-NONEXECUTABLE-COPY.pdf)
1005	Wright, T.R. et al., Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes, PNAS 107 (47) 20240-20245 (11/23/2010) (https://doi.org/10.1073/pnas.1013154107)
1006	*Enlist™ Herbicide Tolerance and Soybean Symptomology - Get the Facts* (https://www.enlist.com/en/News/enlist-tolerance-soybean-symptomology.html) (7/13/2021)
1010	Declaration Under 37 C.F.R. §1.132 of Dr. Terry R. Wright (7/29/2021)
1011	Corteva Agriscience: Enlist 1 Herbicide Label (https://www.corteva.ca/content/dam/dpagco/corteva/na/ca/en/files/products/label/DF-Enlist-1-Herbicide-Label-English.pdf)
1012	Corteva Agriscience: Enlist Duo Herbicide Label (https://www.corteva.ca/content/dam/dpagco/corteva/na/ca/en/files/products/label/DF-Enlist-Duo-Herbicide-Label-English.pdf)
1013	Anjanappa, R.B. and Gruissem, W., 2021. Current progress and challenges in crop genetic transformation. Journal of Plant Physiology, 261, 153411.
1014	Herman, P.L., Behrens, M., Chakraborty, S., Chrastil, B.M., Barycki, J. and Weeks, D.P., 2005. A three-component dicamba O-demethylase from Pseudomonas maltophilia, strain DI-6: gene isolation, characterization, and heterologous expression. *Journal of Biological Chemistry*, *280*(26), pp.24759-24767
1015	Chekan, Jonathan R, et al. "Molecular basis for enantioselective herbicide degradation imparted by aryloxyalkanoate dioxygenases in transgenic plants." Proceedings of the National Academy of Sciences 116.27 (2019): 13299-13304.
1016	Englander, S.W. and Mayne, L., 2014. The nature of protein folding pathways. Proceedings of the National Academy of Sciences, 111(45), pp.15873-15880.

1017	Gao SS, Naowarojna N, Cheng R, Liu X, Liu P. Recent Examples of α-Ketoglutarate-Dependent Mononuclear Non-Haem Iron Enzymes in Natural Product Biosyntheses. Nat Prod Rep. 2018 Aug. 15; 35(8): 792–837.
1018	Peach C, Velten J. Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters. Plant Mol Biol. 1991;17(1):49-60.
1019	Hogan, D.A., Smith, S.R., Saari, E.A., McCracken, J. and Hausinger, R.P., 2000. Site-directed Mutagenesis of 2, 4-Dichlorophenoxyacetic Acid/α-Ketoglutarate Dioxygenase: IDENTIFICATION OF RESIDUES INVOLVED IN METALLOCENTER FORMATION AND SUBSTRATE BINDING. Journal of Biological Chemistry, 275(17), pp.12400-12409.
1020	Müller, T.A., Zavodszky, M.I., Feig, M., Kuhn, L.A. and Hausinger, R.P., 2006. Structural basis for the enantiospecificities of R- and S-specific phenoxypropionate/α-ketoglutarate dioxygenases. Protein Science, 15(6), pp.1356-1368.
1021	Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. Nature. 2021 Aug.;596(7873):583-589.
1022	Bagdonas, H., Fogarty, C.A., Fadda, E. and Agirre, J., 2021. The case for post-predictional modifications in the AlphaFold Protein Structure Database. Nature Structural & Molecular Biology, 28(11), pp.869-870.
1023	Müller, TA, Fleischmann, T, van der Meer, JR and Kohler, HPE, Purification and characterization of two enantioselective α-ketoglutarate-dependent dioxygenases, RdpA and SdpA, from Sphingomonas herbicidovorans MH. Applied and environmental microbiology, 2006. 72(7), pp.4853-4861.
1024	Prabakaran, S., Lippens, G., Steen, H. and Gunawardena, J., 2012. Post-translational modification: nature's escape from genetic imprisonment and the basis for dynamic information

	encoding. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 4(6), pp.565-583.
1025	Streber, W.R., Timmis, K.N. and Zenk, M.H., 1987. Analysis, cloning, and high-level expression of 2, 4-dichlorophenoxyacetate monooxygenase gene tfdA of Alcaligenes eutrophus JMP134. Journal of Bacteriology, 169(7), pp.2950-2955.
1026	Brannigan, J.A. and Wilkinson, A.J., 2002. Protein engineering 20 years on. Nature Reviews Molecular Cell Biology, 3(12), pp.964-970.
1027	Westendorf, A, Müller, RH, Babel, W, Purification and characterisation of the enantiospecific dioxygenases from Delftia acidovorans MC1 initiating the degradation of phenoxypropionate and phenoxyacetate herbicides. Acta biotechnologica, 2003. 23(1), pp.3-17.
1028	Levels of Protein Organization (https://comis.med.uvm.edu/VIC/coursefiles/MD540/MD540-Protein_Organization_10400_574581210/Protein-org/index.html)
1029	Corteva Agriscience Enlist Weed Control System 2020 Product Use Guide (https://www.enlist.com/content/dam/hdas/enlist/pdfs/Enlist_Product_Use_Guide_2020.pdf)
1033	Nelson, D.L, and Cox, M.M., 2005. Lehninger Principles of Biochemistry, 4th edition, pp.116-156.
1034	Mall, T., Gupta, M., Dhadialla, T.S. and Rodrigo, S., 2019. Overview of biotechnology-derived herbicide tolerance and insect resistance traits in plant agriculture. Transgenic plants: methods and protocols, pp.313-342.