

In the Matter of:

*Inari Agriculture, Inc. vs
Corteva Agriscience LLC*

Paul Christou, Ph.D.

March 05, 2024

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4 UNITED STATES PATENT AND TRADEMARK OFFICE

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6 BEFORE THE PATENT TRIAL AND APPEAL BOARD

7
8 INARI AGRICULTURE, INC.

9 Petitioner,

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11 V.

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13 CORTEVA AGRISCIENCE, LLC,

14 Patent Owner.

15
16 Case PGR 2023-00022

17 Patent No. 11,371,955
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- - -
Tuesday, March 5, 2024
- - -

Deposition of PAUL CHRISTOU, PH.D. taken
at Finnegan, Henderson, Farabow, Garrett & Dunner,
LLP, 901 New York Avenue, NW, Washington, DC
commencing at 9:21 a.m., before Sherry L. Brooks,
Certified LiveNote Reporter and Notary Public, in and
for the District of Columbia.

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I N D E X

- - -

TESTIMONY OF: Paul Christou, Ph.D.

By Mr. Rutt 6

By Mr. Flibbert 147

P R E V I O U S L Y M A R K E D E X H I B I T S

EXHIBIT NUMBER	DESCRIPTION	PAGE MARKED
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Exhibit 2012	Declaration of Paul Christou, Ph.D. - Inari Agriculture, Inc. -v- Corteva Agriscience, LLC	9
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Exhibit 2057	Genetic Engineering Principles and Methods - Edited by Jane K. Setlow	13
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Exhibit 1001	United States Patent, Wright Patent No. 11,371,055 B2	19
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Exhibit 2076	Plant Physiology - Structure of a Vicilin Protein	30
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EXHIBITS CONTINUED:

P R E V I O U S L Y M A R K E D E X H I B I T S

EXHIBIT NUMBER	DESCRIPTION	PAGE MARKED
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Exhibit 1043	Transformation and Regeneration of Two Cultivars of Pea	31
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Exhibit 2079	Plant Cell, Tissue and Organ Culture - An International Journal on the Cell Biology of Higher Plants	50
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Exhibit 1044	The Quest to Understand the Basis and Mechanisms that Control Expression of Introduced Transgenes in Crop Plants	108
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(Exhibits attached to transcript.)

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P R O C E E D I N G S

* * * * *

WHEREUPON,

PAUL CHRISTOU, PH.D.

after having been first duly sworn, was
examined and testified as follows:

- - -

EXAMINATION BY COUNSEL FOR PETITIONER

BY MR. RUTT:

Q. Dr. Christou, nice to meet you. My name
is Eric Rutt. I'm from Wolf Greenfield and I'm
representing Inari today.

So I'm going to be asking you some
questions and you're going to do your best to answer
them, right?

A. For sure, I will.

Q. If you have any need for a break, just let
me know. I just ask that if there's a question
that's pending that you answer the question first.
If anything is unclear, let me know. I'll try to
clarify it for you.

A. Thank you.

1 Q. Have you ever been deposed before?

2 A. Yes, I have.

3 Q. When was that?

4 A. I would say over ten years ago, maybe
5 closer to 15 years ago.

6 Q. And what was the nature of the proceeding?

7 A. What was the nature of the proceeding? It
8 was a case involving transformation, plant
9 transformation, which is my area of expertise. But
10 I'm afraid I don't recall the details.

11 Q. Were you testifying as an expert witness?

12 A. Yes, I was.

13 Q. And who hired you?

14 A. It was a case involving Finnegan. But,
15 again, I'm drawing a blank. I'm sorry. I don't
16 remember.

17 Q. Was it in the United States?

18 A. It was in the United States, yes.

19 Q. Did it involve patents?

20 A. It involved patents.

21 Q. When you were retained by Corteva, who
22 reached out to you initially?

1 A. Originally, attorneys from Finnegan.

2 Q. And then after that was there somebody
3 else?

4 A. No. It was always attorneys from
5 Finnegan.

6 Q. And about how long did you spend preparing
7 for this deposition today?

8 A. Are you referring to the whole time I
9 spent on the case or just preparing for the
10 deposition today?

11 Q. Just for today.

12 A. Just for today, I would say about ten to
13 12 hours.

14 Q. And when did you prepare?

15 A. Well, I flew in from Europe on Saturday
16 afternoon. We had a meeting here on Sunday for four
17 or five hours, the same yesterday, and that's about
18 it.

19 Q. And who did you meet with at that meeting?

20 A. With Mr. Flibbert and Ms. Yang and with
21 Pier DeRoo.

22 Q. And did you review any documents during

1 that meeting?

2 A. I did.

3 Q. which documents did you review?

4 A. We focused on the '055 patent and my
5 expert report.

6 Q. Any other documents?

7 A. Yes. We looked at other documents as
8 well, but not in great detail.

9 Q. which ones did you look at?

10 A. I -- we looked at Dr. Silverstone's
11 declaration, a few scientific papers I cited in my
12 expert report.

13 Q. So Dr. Christou, I'll show you a document
14 marked Exhibit 2012.

15 A. Thank you.

16 Q. Do you recognize this document?

17 A. I recognize the front page.

18 Q. why don't you look through it and see if
19 it appears to be your declaration?

20 A. Yes, it appears to be my declaration.

21 Q. So turn in your declaration to Appendix B
22 at the end.

1 A. Okay.

2 Q. You mentioned you had looked at several
3 scientific documents in your preparation for today.
4 Do you recognize any of those documents on this list?

5 A. I recognize the first one, the second one.
6 The third one I'm not sure. I recognize Paine, et
7 al.

8 Q. Let me pause right there. When you say
9 "recognize," do you mean you looked at those
10 yesterday and the day before?

11 A. I looked at the '055 patent and the -- Dr.
12 Silverstone's declaration. I did not look at the
13 Paine, et al. paper yesterday.

14 Q. Why don't you go through this list and let
15 me know which exhibit numbers you looked at?

16 A. I looked at Exhibit No. 2057. I looked at
17 -- very briefly at Exhibit 2062 Potenza Cahol (sic).

18 MR. FLIBBERT: And, Dr. Christou, I would
19 just caution you not to reveal the substance of any
20 communications with counsel. But you can answer the
21 question.

22 A. I recall glancing at 2065, Maria Tal

1 (sic). I believe these are the ones I looked at.

2 BY MR. RUTT:

3 Q. Did you write this declaration yourself?

4 A. Well, this was an iterative process. So
5 we had Zoom meetings. I would be asked -- I was
6 asked to review certain documents and I was asked to
7 give opinions pertaining to my area of expertise,
8 which is plant transformation.

9 I had to do a literature search to
10 identify documents which I felt were relevant to the
11 case and then I would explain my opinion to Finnegan
12 attorneys, and then they would present me with a
13 draft of what I said. I would look at it, make sure
14 that it reflected my opinion.

15 Then we went through this a number of
16 times and this is how the report was created. So, in
17 essence, even though I did not handwrite the report
18 myself or type it myself, it reflects pretty much
19 everything I did and everything I said.

20 Q. And about how many hours did you spend
21 working with Finnegan to prepare your declaration?

22 A. It will be a guess. I'll try and make it

1 as accurate as I can. I would say between 20 or 30
2 hours within that period excluding -- I mean, writing
3 the report, yes.

4 Q. And what are you billing an hour for this
5 case?

6 A. My standard rate, which is \$525.

7 Q. So about how much have you billed Finnegan
8 for the entire proceeding so far?

9 A. I have to do the math. I don't remember
10 offhand. So I can multiply that by the number of
11 hours. It would be, as I said, between 20 and 30
12 hours.

13 Q. So about \$20,000?

14 A. Well, if that's what the math says, yes.
15 whatever.

16 Q. Turn in your declaration to paragraph 46.
17 So at paragraph 46 it's in a section describing
18 standard plant transformation techniques; is that
19 right?

20 A. That's what the heading says, yes.

21 Q. And in the second sentence in that
22 paragraph you say: "As I explained in the book,

1 'Genetic Engineering Principles and Methods,'
2 published in 2002, gene transfer to a plant was by
3 then 'a routine procedure.'"

4 Do you see that?

5 A. I do.

6 Q. And you cite Exhibit 2057?

7 A. Yes.

8 Q. So I'll hand you what's been marked as
9 Exhibit 2057. Do you recognize this document?

10 A. I do.

11 Q. What is this document?

12 A. This is a book chapter that was written by
13 myself, my Ph.D. student -- one Ph.D. student and two
14 postdoctoral workers in my group when I was working
15 at the John Innes Centre in Norwich in the United
16 Kingdom.

17 Q. Which of the authors was your Ph.D.
18 student?

19 A. The second one.

20 Q. So turn to page 111.

21 A. Okay.

22 Q. Do you see a section at the top entitled

1 T-DNA Integration?

2 A. I do.

3 Q. what is T-DNA?

4 A. T-DNA is a specific segment of DNA in the
5 bacterium, agrobacterium tumefaciens, which
6 scientists developed into a system to introduce
7 heterologous genes into plants.

8 Q. There's a subheading entitled Organization
9 of Transgenic Loci, L-O-C-I.

10 Do you see that?

11 A. Yes, I do.

12 Q. About halfway down that paragraph there's
13 a sentence that begins, "It is generally the case."

14 Do you see that sentence?

15 A. I do.

16 Q. You write in here: "It is generally the
17 case, however, that agrobacterium transformation
18 gives rise to lower transgene copy numbers than
19 direct transformation methods."

20 Do you see that?

21 A. I do.

22 Q. what is a copy number?

1 A. Okay. Does the question refer to this
2 review paper in general in the literature or is it a
3 more specific question so I can understand the
4 context?

5 Q. Do you know what a copy number means?

6 A. In general terms, of course, I do, yes.

7 Q. What is a copy number?

8 A. A copy number is the number of transgene
9 copies that are integrated into the genome of an
10 (inaudible) plant.

11 Q. And why is it that agrobacterium
12 transformation gives rise to a lower copy number?

13 A. This is because of the mechanics of
14 integration of genes that are inserted into plants
15 using agrobacterium. It's the biology of the system.

16 Q. In the next sentence you refer to an
17 "informative experiment."

18 Do you see that?

19 A. I do.

20 Q. What was that informative experiment?

21 A. I don't recall. I need to read it to
22 refresh my memory. Is that okay? Yes. Now, I

1 remember what this is about.

2 Q. If you're able -- in simple terms and in
3 less than a minute -- can you summarize what the
4 informative experiment was about?

5 A. Yes. I mean, the bottom line is that for
6 this specific case -- and I believe the case is wheat
7 transformation -- the Cheng, et al. (phonetic) and
8 coworkers carried out an experiment in which they
9 compare inserting a gene into wheat either using
10 agrobacterium when they did the transformation or
11 direct DNA transfer using particle bombardment.

12 And at the end of the experiment they
13 recovered a population of transgenic plants in each
14 case and then they measured how many copies of the
15 inserted transgene each of the two populations
16 contained. And they came to the conclusion that
17 agrobacterium overall had a lower number of copies of
18 the inserted transgene compared to particle
19 bombardment direct DNA transfer.

20 However, this is not always the case.
21 This is the case in this particular experiment. And
22 I have done experiments myself in a number of

1 different species. And as we have reported in the
2 paper in 2005, direct DNA transfer can also result in
3 a low transgene copy number. So it's not a general
4 theme.

5 Q. So in this one experiment involving wheat
6 there were more single copy numbers with
7 agrobacterium transfer than with particle
8 bombardment; is that right?

9 A. That is correct for this specific example.
10 But I do recall that I read other papers involving
11 wheat which the situation was not as described in
12 this paper. And, again, this is not surprising.
13 It's a general rule in plant transformation.

14 Q. So the copy number can depend on the
15 transformation technique. Is that fair to say?

16 A. No. This is not what I meant. I meant
17 that different papers in the literature on the same
18 species may or may not report the same result, and
19 this is some of the issues that we have to deal with
20 in the literature where some papers say one thing and
21 others say another.

22 Q. Why is the literature so confusing and

1 contradictory?

2 MR. FLIBBERT: Objection to form.

3 Mischaracterizes testimony.

4 A. Could you please repeat the question?

5 BY MR. RUTT:

6 Q. Why is the literature so confusing and
7 contradictory?

8 MR. FLIBBERT: Same objections.

9 A. I wouldn't say the literature is confusing
10 in any way. It's just that in this particular paper,
11 for example, the data went one way and another went a
12 different way.

13 However, if we look at the literature, the
14 general literature overall, we have a pretty good
15 idea depending on what a specific transgene is,
16 whether one technique or another is going to behave
17 differently in a different crop.

18 BY MR. RUTT:

19 Q. So you're saying you can predict the copy
20 number if you know the species that's being targeted?

21 MR. FLIBBERT: Objection to form.

22 A. Well, I mean, I know this discussion is

1 about copy number. However, and this -- I've
2 published this in many of my papers and other people
3 have done the same.

4 Copy number is not really relevant in
5 terms of whether a transgene would express well or
6 not in a given species irrespective of the method of
7 transformation.

8 Q. So I'll show you a document marked Exhibit
9 1001. Do you recognize this document?

10 A. Yes, I do.

11 Q. What is this document?

12 A. It's the '055 patent.

13 Q. So turn in this document to column 28,
14 line 37. And just read silently to yourself lines 37
15 through 44.

16 A. Yes.

17 Q. So it's saying here that copy number can
18 increase the expression of a given gene, right?

19 A. This is what it says, yes.

20 Q. So would you agree with that statement?

21 A. I would say it can. So within the context
22 of this section I've read I would agree with the

1 statement with caveats.

2 Q. I mean, you had said that copy number is
3 not really relevant in terms of whether a transgene
4 would express well or not in a given species,
5 correct?

6 A. That is correct.

7 Q. But here it says that transgene expression
8 is effective by copy number, right?

9 A. well, it doesn't exactly say "is." It
10 says "can." And there is a big difference
11 technically between "is" and "can," in my mind.

12 Q. But you had said copy not is not relevant,
13 right, to expression?

14 A. That was in the context of your earlier
15 question. In general, there may be cases where copy
16 number, especially the architecture of the transgenic
17 locus, is in a way that will be conducive to
18 increased expression on occasion.

19 Increased copy number, as long as they're
20 in a specific orientation, may result in increased
21 levels of expression, but it's not an all-or-nothing
22 occurrence.

1 Q. So sometimes copy number is relevant to
2 gene expression?

3 A. In general terms it may be relevant. But,
4 again, since you are talking about the '055 patent,
5 my impression is that it may not be that relevant
6 because what we are looking at here is the resulting
7 phenotype of the plants which would express the
8 introduced trait. And as long as they express the
9 introduced trait, the copy number becomes irrelevant.

10 Q. So does copy number never have an effect
11 on the trait?

12 A. This is not what I said. It's that on
13 occasion if the architecture of the transgenic locus
14 is conducive it may have an effect.

15 Q. And do you believe that with the AAD-12
16 gene it will not -- the copy number will not have an
17 effect on transgene expression?

18 A. I simply don't know because I haven't
19 considered that. What I considered was whether the
20 resulting plant, transgenic plants, would express the
21 introduced transgene. And, indeed, they do. I
22 haven't considered copy number because personally I

1 don't really care about copy number.

2 Q. Let's go back to Exhibit 2057. So turn to
3 page 112.

4 A. Okay.

5 Q. Do you see the section entitled Transgene
6 Rearrangement?

7 A. I do.

8 Q. And just above that heading there's a
9 sentence that says: "Transgene locus structure may
10 be extremely sensitive to variations of a particular
11 transformation method."

12 Do you see that?

13 A. Yes, I do.

14 Q. What is transgene locus structure?

15 A. In general terms?

16 Q. Yeah.

17 A. Okay. A transgene locus is the position
18 in the genome of a plant where a given number of
19 transgenes copy integrate and on top of that the way
20 different copies are arranged within that locus.

21 Q. Why is the locus extremely sensitive to
22 variations of a particular transformation method?

1 MR. FLIBBERT: Objection to form.

2 A. Can you please repeat the question?

3 BY MR. RUTT:

4 Q. why is transgene locus structure extremely
5 sensitive to variations of a particular
6 transformation method?

7 MR. FLIBBERT: Same objection.

8 A. It is not sensitive to a particular
9 transformation method because it can happen
10 irrespectively over transformation methods. So we
11 are talking about transgene locus, and the transgene
12 locus can be formed irrespectively of the
13 transformation method. The transformation method is
14 the method we use to introduce transgenes.

15 Now, from that point on the formation of
16 the locus is not really dependent on how the
17 transgenes are arranged or the copies of the
18 transgenes are not dependent on the method.

19 BY MR. RUTT:

20 Q. I'm just reading what you wrote here. It
21 says: "Transgene locus structure may be extremely
22 sensitive to variations of a particular

1 transformation method."

2 Did I read that correctly?

3 A. Yes. By this, it means by particular
4 transformation method. It doesn't necessarily mean
5 that we are making a distinction between
6 agrobacterium and particle bombardment.

7 Q. I didn't ask about that. I'm just asking
8 if you agree that transgene locus structure may be
9 extremely sensitive to variations of a particular
10 transformation method?

11 MR. FLIBBERT: Objection. Asked and
12 answered.

13 A. I mean, maybe I'm misunderstanding the
14 question, and please help me out if I do. But I
15 think what I said -- what I said is that the
16 organization and the structure of the locus in its
17 complexity is something that happens after the
18 transformation.

19 And I think I also mentioned that
20 irrespectively of whether one uses agrobacterium or
21 direct DNA transfer the locus of the structure would
22 assume a certain complexity.

1 Q. So are you disagreeing with this sentence
2 that we read?

3 A. No, I'm not disagreeing with the sentence.

4 Q. Okay. So I'm asking what makes, in some
5 cases, the transgene locus extremely sensitive?

6 A. The major factor is the arrangement of
7 different copies of transgenes in the locus. Once a
8 transgene or several copies of a transgene integrate
9 into the locus they can be arranged in a number of
10 different ways.

11 They may be arranged in what we call a
12 head-to-tail orientation, where the transcription
13 will be opposite. And this is not very conducive for
14 transgene expression, or they may be arranged in what
15 we refer to as concatemeric repeats, which means that
16 they are transcribed in the same orientation.

17 So if it's the latter, then the expression
18 is expected to be higher than if the arrangement was
19 different.

20 Q. Let me make sure I understand. So in
21 simple terms, head-to-tail is good; head-to-head is
22 bad.

1 Is that fair?

2 MR. FLIBBERT: Objection to form.

3 A. I believe what I said is that if the
4 arrangement of transgenes is in such a way that they
5 form concatemeric repeats. So this is conducive for
6 good expression.

7 BY MR. RUTT:

8 Q. Head-to-tail is conducive to good
9 expression; is that right?

10 A. If they all transcribe in the same
11 orientation, yes.

12 Q. So if all the concatemers are
13 head-to-tail, that is good for gene expression; is
14 that right?

15 A. One concatemer comprising of multiple
16 copies.

17 Q. Okay. So one concatemer with all
18 head-to-tail orientation is good for gene expression;
19 is that right?

20 MR. FLIBBERT: Objection to form.

21 A. I mean in general terms, of course. But,
22 again, this is also gene dependent.

1 BY MR. RUTT:

2 Q. Is it true in the case of AAD-12?

3 A. I mean, I don't know because I have not
4 looked at the structure of the transgenic loci. And,
5 again, I think I mentioned this already and I repeat
6 it and I reiterate that it doesn't really matter
7 because what we care about is whether the phenotype
8 we expect out of a transformation event manifests
9 itself or not.

10 And as long as it does, personally I
11 really don't consider at all what the copy number or
12 what the locus organization is. For me, it's
13 irrelevant.

14 Q. And head-to-head orientation within a
15 concatemer is in general bad for gene expression; is
16 that right?

17 MR. FLIBBERT: Objection to form.

18 A. Can you please repeat the question?

19 BY MR. RUTT:

20 Q. Head-to-tail (sic) concatemer orientation
21 is generally bad for gene expression, right?

22 MR. FLIBBERT: Objection to form.

1 A. I mean, head-to-tail they are all
2 transcribed in the same direction. It's not
3 head-to-head, so I think we talked about that.

4 BY MR. RUTT:

5 Q. Maybe I misspoke -- sorry. Head-to-head
6 concatemer orientation is generally bad for gene
7 expression; is that right?

8 MR. FLIBBERT: Objection to form.

9 A. It may be bad for gene expression. But,
10 again, in the case -- in a practical situation it's
11 not really relevant because we don't look for those
12 kind of plants in a transformation experiment.

13 They are selected out at the early stage
14 of the transformation process. So, again, this is
15 what I tell my students, don't waste your time
16 looking at transgene copy number, transgene
17 organization. Go straight for the phenotype.

18 And if you get the phenotype, you don't
19 need to waste your time to actually determine what
20 the structure of the locus is or not, because if you
21 have a phenotype that shows good expression, that's
22 really what matters, irrespective of the organization

1 of the transgenic locus.

2 BY MR. RUTT:

3 Q. And when you say "that's really what
4 matters," what do you mean? That's what matters for
5 sort of using the transgene in the real world?

6 A. I mean, it depends on what "real world"
7 means. I think it's pretty general.

8 Q. You said it's what matters. I'm trying to
9 understand what you mean by matters. Matters for
10 what?

11 A. It matters in terms of getting an answer
12 out of a given experiment. So this is what I mean.

13 Q. Okay. So in scenarios where, say, there
14 are problems and there's no phenotypic expression,
15 are you saying those are the sorts of scenarios we're
16 not interested in; we only care about scenarios where
17 there is phenotypic expression?

18 MR. FLIBBERT: Objection to form.

19 A. And I don't believe this is what I said.
20 I think I restricted my answer to a given experiment.
21 So my assumption in answering your question -- and
22 please correct me if I make the wrong assumption --

1 is that we have a transgene that we know that
2 expresses and it results in a given phenotype.

3 So what I was talking about was a
4 population of plants derived from that experiment.
5 And, obviously, that population of plants would
6 exhibit variation and levels of gene expression from
7 -- at various levels.

8 And what the selection process in the
9 transformation -- what the selection component of the
10 transformation process is meant to do is to weed out
11 the events that are not really performing as the
12 experimenter wished them to perform.

13 And, therefore, this selection is
14 extremely important to allow us to derive a
15 population of plants which would be the plants to
16 focus on in subsequent experiments.

17 BY MR. RUTT:

18 Q. I think it might help to look at a
19 specific experiment since we've been talking in
20 abstraction. So I'm going to hand you Exhibit 2076.

21 A. Thank you.

22 Q. Do you recognize this document?

1 A. I'm afraid I do not.

2 Q. So --

3 A. Excuse me. What is the year of
4 publication, if I may ask -- oh, sorry. I found it,
5 1993.

6 Q. So in your declaration on paragraph 51 you
7 cite this exhibit.

8 A. Okay. Where did I cite it? Could you
9 please remind me?

10 Q. Paragraph 51.

11 A. I surely did. I forgot. I'm sorry.

12 Q. Does this document appear to be the
13 document you cited in your declaration?

14 A. Yes, it does.

15 Q. And I had a difficult time reading it
16 because it's so tiny, so I'm handing you a new
17 exhibit marked 1043.

18 A. Thank you.

19 Q. Does this appear to be the same article
20 that is in Exhibit 2076?

21 A. Yes, it does.

22 Q. And the title of this article is

1 Transformation and Regeneration of Two Cultivars of
2 Pea, P-E-A.

3 Do you see that?

4 A. I do.

5 Q. In the abstract in the second sentence, it
6 states: "A construct containing two chimeric,
7 C-H-I-M-E-R-I-C, genes."

8 Do you see that?

9 A. I do.

10 Q. what is a chimeric gene?

11 A. In general terms, a chimeric gene is a
12 term which is commonly used in the field which
13 describes a gene that is joined to different
14 components which come from different organisms.

15 Q. And what is a construct?

16 A. A construct is a sequence of DNA which
17 contains, in addition to the gene of interest, other
18 components that may facilitate the transformation
19 process in different ways.

20 Q. So at the bottom of the first page there,
21 page 751 -- actually, strike that -- the next page,
22 752, do you see a section entitled Plant

1 Regeneration?

2 A. I do.

3 Q. And in the second sentence it refers to
4 explants that were placed onto a callus, C-A-L-L-U-S.

5 A. Sorry. I lost you.

6 Q. The Plant Regeneration section, second
7 sentence.

8 A. Okay. Starting with, "Excess liquid"?

9 Q. That's right. And it refers to "explants
10 were placed onto a callus induction medium."

11 Do you see that?

12 A. I do.

13 Q. what is an explant?

14 A. An explant is a part of the plant that is
15 removed from the plant to be used in a given
16 transformation experiment.

17 Q. And what is a callus?

18 A. A callus is a differentiated mass of cells
19 that are derived from an explant once the explant is
20 placed on media with certain plant hormones to induce
21 cell differentiation.

22 Q. In the sentence after that one, it refers

1 to PPT.

2 Do you see that?

3 A. Yes, I do.

4 Q. What is PPT in general terms?

5 A. PPT stands for phosphinothricin.

6 Q. I'm just curious what it is in a really
7 general term.

8 A. It's a compound. It's an herbicide that
9 is used as a selectable marker in certain
10 transformation experiments.

11 Q. So in the next paragraph in that section
12 on plant regeneration, it describes transferring the
13 explants to a P3 medium.

14 Do you see that?

15 A. Yes, I do.

16 Q. Why are they transferring the explants to
17 a different medium in this experiment, if you know?

18 A. I need to read the entire section to
19 understand what P1, P2, and P3 media are. May I?

20 Q. Please do.

21 A. Okay. Thank you. I get it. Now, I
22 understand.

1 Q. In a really general understanding, why did
2 they transfer to a different medium in this step?

3 A. The P3 medium, as I read here, is exactly
4 the same as P2 medium, with the only difference that
5 P3 medium also contains the selectable marker.

6 Q. And what's the purpose of doing this?

7 A. The reason for doing this is application
8 of selection to actually distinguish between tissues
9 that have been transformed and contain the input gene
10 and those that have not.

11 Q. And in this case the input gene appears to
12 be the bar gene; is that right?

13 A. I mean, this is what I assume, but I don't
14 know. I haven't read the rest of the paper and I
15 don't know if there is any other gene mentioned.
16 But, obviously, PPT is being detoxified by the bar
17 gene.

18 Q. In the right column at the top of that
19 page, 752, do you see a section entitled Bacterial
20 Strain and Gene Construction?

21 A. I do.

22 Q. There it describes the bar gene and the

1 nptII gene, right?

2 A. Yes.

3 Q. So were these the two genes that were
4 being input?

5 A. These are the two genes that I referred
6 to. So I presume, yes, these are the two genes.

7 Q. In the next section entitled Enzyme Assays
8 it refers to PAT activity.

9 Do you see that?

10 A. I do.

11 Q. What is PAT activity?

12 A. It's the enzymatic activity which the
13 transgenic plants express to detoxify the herbicides,
14 in this case for phosphinothricin, PPT.

15 Q. So PAT is what degrades PPT; is that
16 right?

17 A. That is right.

18 Q. And so in this experiment the researchers
19 were testing for PAT activity in the plants that had
20 been transformed; is that right?

21 A. Indeed, they were testing for enzymatic
22 activity in the plants.

1 Q. And at the bottom of that section, the
2 last sentence says: "NPT was detected."

3 Do you see that?

4 A. I don't remember what NPT means, I'm
5 afraid. Is there a key or an abbreviation list I can
6 look at?

7 Q. Might it refer to the nptII gene?

8 A. Yes. Yes. As a matter of fact, I see it
9 up under Bacterial Stain and Gene Construction. I'm
10 sorry I missed that.

11 Q. Also in this section in the sentence --
12 second to last sentence it refers to C, superscript
13 14, labeled compounds.

14 Do you see that?

15 A. Yes, I do.

16 Q. what is a C14 labeled compound?

17 A. well, it's -- C14 is an isotope of carbon,
18 which is -- which was being used at the time -- no
19 longer because we have much better methods now -- to
20 detect enzymatic activity.

21 Q. So turn to page 754 of this article. In
22 the section on results, about the third sentence from

1 the end of the first paragraph, it starts with the
2 phrase, "The time required."

3 Do you see that?

4 A. Yes, I do.

5 Q. And it says: "The time required to
6 progress from the explant shown in figure 1a to the
7 mature plant in figure 1g was approximately nine
8 months."

9 Do you see that?

10 A. Yeah. May I see what figures 1a and 1g
11 refer to, please?

12 Q. Sure.

13 A. Yes, I see.

14 Q. Is that a typical length of time that's
15 required in these sorts of experiments?

16 MR. FLIBBERT: Objection to form.

17 A. I guess referring to pea transformation it
18 must be because this is a paper on peas, the plant.

19 BY MR. RUTT:

20 Q. Staying with that paragraph, the last
21 sentence says: "Between 1.5 and 2.5 percent of the
22 starting explants gave rise to transformed plants."

1 Do you see that?

2 A. I do.

3 Q. what does that mean?

4 A. I mean, this is typically how the
5 frequency or the efficiency of a transformation
6 experiment is expressed. So the authors here are
7 telling their readers how good their transformation
8 experiment was.

9 Q. Are these good results?

10 MR. FLIBBERT: Objection to form.

11 A. I mean, I don't know. I'm not working on
12 peas. So I don't know if this is good for peas. I'm
13 sorry, I don't know.

14 BY MR. RUTT:

15 Q. what kinds of species did you work on?

16 A. Rice, corn, soya -- soybean.

17 Q. S-O-Y-A?

18 A. S-O-Y-A. We can drop the A, soy.

19 Q. Is 1.5 to 2.5 percent transformation rate
20 good for rice?

21 A. No. That's a terrible transformation rate
22 for rice.

1 Q. How about for soybeans?

2 MR. FLIBBERT: Objection to form.

3 A. I don't recall. But if I go back to my
4 days when I was doing soya transformation 40 years
5 ago, I would have been very happy with a
6 transformation frequency of 1.5 to 2.5 percent for
7 soya.

8 BY MR. RUTT:

9 Q. Does that mean that 98 to 97 percent of
10 the plants were not having -- not being transformed?

11 A. I will turn it around because this is how
12 we work in my field. I would not say how many were
13 not transformed because you don't care about those,
14 and those are eliminated in the selection.

15 So I would say the outcome of the
16 experiment was the recovery of 1.5 to 2.5 percent of
17 plants because we really don't even know how many
18 plants you would get because there they would not
19 even regenerate under selection. So what you get in
20 the end of a transformation experiment is plants that
21 are transformed.

22 So this is the number you get. I mean,

1 any known transformed potential plant would not
2 survive the selection process. So this is how it
3 works.

4 Q. In the next paragraph the very last line
5 on the left column refers to PAT activity that was
6 detected in eight of the nine plants.

7 Do you see that?

8 A. I do.

9 Q. And it refers to figure 2.

10 A. Yes.

11 Q. So go ahead and read silently to yourself
12 the caption in figure 2 and familiarize yourself with
13 figure 2 so we can discuss it.

14 MR. RUTT: Maybe this would be a good time
15 for a break. I see we've been going for an hour, so
16 why don't we take a break.

17 (A break was taken.)

18 BY MR. RUTT:

19 Q. Welcome back, Dr. Christou. During your
20 break did you discuss the substance of your testimony
21 with your counsel?

22 A. No, I did not.

1 Q. We're going to talk about a lot of
2 technical terms today. So if possible, if you could
3 break your answers into smaller segments that would
4 be helpful for me and the court reporter.

5 A. I will certainly do that.

6 Q. Okay. We were talking about Exhibit 1043,
7 page 754, figure 2.

8 Do you recall that?

9 A. I do.

10 Q. What does figure 2 show?

11 A. It shows the results of an enzymatic assay
12 for PAT activity on leaf extracts from transgenic pea
13 plants.

14 Q. What does the single asterisk indicate?

15 A. That is -- it denotes the substrate for
16 the enzymatic reaction.

17 Q. And what does the double asterisk
18 indicate?

19 A. That is the product of the enzymatic
20 reaction.

21 Q. So the top line is substrate, the bottom
22 line is the product that resulted. Is that what it's

1 showing?

2 A. This is what it's showing, yes.

3 Q. And the first lane is a positive control
4 that just shows the product that results when the bar
5 gene is present, right?

6 A. Yes. It's an extract from a
7 pre-transformed transgenic tobacco plant expressing
8 the bar gene.

9 Q. And lane 2 is the negative control, which
10 is from a plant that has not been transformed, right?

11 A. That is correct.

12 Q. And then what is in lane 13? I can read
13 it, but I don't know what it means.

14 A. No. It's a technical control to show that
15 in the absence of the plant extract from a transgenic
16 plant, I presume, there is no reaction. So it's a
17 control to make sure that the experimental conditions
18 are appropriate.

19 Q. And then lanes 3 through 12 show the
20 results of the nine transformed plants; is that
21 right?

22 A. That is correct.

1 Q. Lanes 4 and 9 appear to be -- to have no
2 product, right?

3 A. That is correct.

4 Q. why does that happen?

5 A. This is very normal in any transformation
6 experiment. Some transgenic plants may express the
7 transgene and others may not.

8 Q. Do they all have the identical gene of
9 interest?

10 A. I need to check and see if there is nptII
11 activity in each and every one of these plants to be
12 able to answer the question.

13 Q. But the intention of the experiment was to
14 put the same genetic sequence into these nine plants;
15 is that right?

16 A. well, that was the intent.

17 Q. And then in lane 7 it looks like -- well,
18 how would you interpret what happened in lane 7?

19 A. I see that all the substrate is gone.

20 Q. what does that mean in simple layperson
21 language?

22 A. It means that this specific plant is a

1 plant that I would personally consider to be my
2 candidate plant to develop further because it appears
3 to have very high enzymatic activity.

4 So in my own experiments when I see
5 something like this, I will discard all other plants
6 and focus on Number 7.

7 Q. What about Number 12? That's pretty good,
8 too, right?

9 A. That's pretty good, too. So I may hedge
10 my bets and keep that as well. But my focus would be
11 on Number 7.

12 Q. In this experiment the researchers were
13 trying to insert the same gene into these pea plants,
14 right?

15 A. Correct.

16 Q. So the difference in the product amounts
17 is not because there's a different DNA input
18 sequence, right?

19 A. Of course not.

20 Q. Did -- do you think the input DNA was
21 added to the same location in the plant genome for
22 all nine?

1 A. Not at all. They would be completely
2 random.

3 Q. why is that?

4 A. Because the mechanics of DNA integration
5 of foreign transgenes into plants is random. We
6 don't have any control of where in the genome a gene
7 would insert.

8 Q. Can you control it with other techniques
9 besides agrobacterium? Can you control the locus
10 using other techniques besides agrobacterium?

11 A. I wish. Unfortunately, we cannot. It's
12 one of the holy grails of plant biotechnology.

13 Q. Is it possible that some of the variation
14 in product here was a result of the location where
15 the DNA was input into these nine plants?

16 MR. FLIBBERT: Objection. Form.

17 A. It may be possible, but I consider it
18 irrelevant.

19 BY MR. RUTT:

20 Q. why do you consider it irrelevant?

21 A. I consider it irrelevant because -- again,
22 I think I mentioned this in response to a past

1 question.

2 what we care about is whether we have a
3 plant or a number of plants that express the
4 phenotype we want. And in this specific case the
5 phenotype would be represented by -- in my mind by
6 plants Number 7 and plants Number 12 as being
7 probably the best plants that come out of this
8 experiment.

9 So I really would not consider the other
10 plants. They're really not that relevant.

11 Q. In the right column on this page in the
12 top paragraph, the last sentence refers to the level
13 of PAT enzyme activity varying by about 20-fold.

14 Do you see that?

15 A. I do.

16 Q. Is that typical or unusual for the amount
17 of variation in the level of the expressed --

18 MR. FLIBBERT: Objection to form.

19 A. I mean, it's neither surprising, nor
20 unusual. Because we cannot control the way they
21 attach and will insert, it's normal to see these kind
22 of variations. And you see it in all genes in every

1 case.

2 BY MR. RUTT:

3 Q. In lane 9 I can't see anything in the
4 product, can you?

5 A. We are talking about figure 2 still?

6 Q. That's right.

7 A. That is correct.

8 Q. In the paragraph in the right column that
9 we were just looking at, it says only a small
10 proportion was converted in that lane.

11 Do you see that?

12 A. I do.

13 Q. How do they know that?

14 MR. FLIBBERT: Objection to form.

15 A. I mean, to be honest, I don't know how
16 they know that. I mean, I don't know that.

17 BY MR. RUTT:

18 Q. Lane 4 and Lane 9 look the same, right?

19 A. You're testing my eyes. There may be a
20 smudge in lane 9. I'm not sure. But, again, is this
21 relevant? It's not relevant in my mind.

22 Q. Is it possible that there was some small

1 amount of PAT activity in what's shown in lane 9 that
2 just was below the limit of detection?

3 MR. FLIBBERT: Objection.

4 A. I mean, it may be possible. But, again,
5 in my mind, this is neither here nor there.

6 BY MR. RUTT:

7 Q. Is the limit of detection in these assays
8 ever a concern for researchers like yourself in these
9 kinds of experiments?

10 A. Well, when I see data like this I would
11 say no because most of the plants exhibit activity.
12 So, obviously, there is no issue of limit of
13 detection here.

14 Q. You're saying that because you're able to
15 see lane 7 and lane 12 are winners. So that's all
16 you care about?

17 A. Indeed.

18 Q. Let's look at your declaration, paragraph
19 55. In the second sentence you give an example of an
20 herbicide resistance gene that was available as a
21 selectable marker.

22 Do you see that?

1 A. I do.

2 Q. And end of that you cite Exhibit 2079.

3 Do you see that?

4 A. I do, yes.

5 Q. I'll show you an exhibit that's been
6 marked as 2079. Does this look familiar to you?

7 A. It does.

8 Q. Are you able to read the font?

9 A. Yes, I can read it.

10 Q. Good, because I didn't have a clear copy
11 of this one. The title of this article is The Use of
12 Glufosinate, G-L-U-F-O-S-I-N-A-T-E, as a Selective
13 Agent in Agrobacterium-Mediated Transformation of
14 Soybean.

15 Do you see that?

16 A. I do.

17 Q. What is glufosinate?

18 A. Glufosinate is an herbicide.

19 Q. And what is it often used for in your
20 field?

21 A. It is used as an agent for selection when
22 the bar or the PAT gene are used.

1 Q. So turn in this exhibit to page 41 and
2 take a moment to review Table 1 and to review as much
3 of the article as you want to be able to discuss it
4 with me.

5 A. But the focus is on Table 1?

6 Q. Table 1. I'd like you to explain the
7 results to me.

8 A. Fine. It's going to take me a while
9 because I need to figure out what the -- it may take
10 me a little bit of time.

11 Q. Take your time.

12 A. Thank you. Right. I think I got the page
13 here. Sorry it took me so long.

14 Q. No problem. So in Table 1, maybe you can
15 explain it in very simple terms why they did three
16 experiments.

17 what was the point?

18 A. At a high level I guess the reason I would
19 do three experiments like they did was to show
20 reproducibility in the results.

21 Q. In experiment 3 they increased the
22 glufosinate concentrations, right?

1 A. They did.

2 Q. But in experiments 1 and 2 they kept them
3 the same, right?

4 A. That is correct.

5 Q. Why would a researcher do that?

6 MR. FLIBBERT: Objection to form.

7 A. To be honest, I don't know. I wouldn't do
8 that.

9 BY MR. RUTT:

10 Q. I couldn't figure it out. I was just
11 curious.

12 A. No. I cannot figure it out. Sorry.

13 Q. Okay. The other thing I noticed about
14 experiments 1 and 2 is that the number of
15 regenerating explants for the non-transformed plants
16 was different.

17 Do you see that?

18 A. I do.

19 Q. In experiment 1 two of the non-transformed
20 plants grew despite having no resistance to the
21 glufosinate, right?

22 A. Yes. This is what I would call escapes.

1 Q. what is an escape?

2 A. An escape is a phenomenon which occurs on
3 occasion -- that takes place on occasion in plant
4 transformation experiments when the -- when a number
5 of plants come through selection, even though they
6 are not transformed because the levels that were used
7 may not have been high enough.

8 And perhaps this answers the earlier
9 question as to why they did -- they increased the
10 levels in experiment 3. But I would say that I
11 encountered similar situations in my experiments with
12 the bar gene in other crops, and this appears to be a
13 peculiarity of the bar gene.

14 Q. So the researchers increased the herbicide
15 and it eliminated those escapes; is that right?

16 A. That is correct. This is what -- this is
17 why selection is so important.

18 Q. Is it possible that by increasing the
19 herbicide the researchers reduced the number of
20 explants that were transformed that were able to
21 regenerate?

22 A. Again, this is very gene-specific. For

1 example, in the case of rice which I'm very familiar
2 with, you get zero escapes with a basal level of
3 selection. As you can see with the bar gene, they
4 increased it a little bit.

5 But, again, it doesn't really matter
6 because in the end what you want is one or two
7 plants, like plants Number 7 and 9 we saw in the
8 earlier paper.

9 Q. So here I was looking at the numbers of
10 regenerating explants in experiments 1, 2, and 3.
11 And I'm just wondering if -- how that number would
12 change if you increased glufosinate to, say, 10
13 milligrams?

14 MR. FLIBBERT: Objection to form.

15 A. I mean, I wouldn't know. I'm sorry.

16 BY MR. RUTT:

17 Q. would it go down?

18 A. It would not go down.

19 Q. what I'm wondering is would -- as you
20 increase glufosinate levels does it begin to kill off
21 even plants -- some of the plants that have been
22 transformed?

1 A. I mean, to answer that question I would
2 need to do what's called a kill curve.

3 Q. It sounds violent.

4 A. No, it's not. It's a standard methodology
5 in plant transformation where before you even start
6 creating transgenic plants you take untransformed
7 wild type tissue and you plate it on different levels
8 of the selective agent, be that antibiotic or
9 herbicide or whatever.

10 And then by doing this you identify a
11 concentration where you get no transformants out of
12 the -- out of the experiment, and then you confirm
13 that in multiple experiments or you are quite sure
14 that this level is the magic level.

15 And then what a practitioner would do is
16 to use that level and -- in normal transformation
17 experiments, and it will be very unusual if you carry
18 out the experiment like that to see escapes.

19 Q. Because now you have a sufficiently high
20 level of herbicide to kill off the escapes?

21 A. To kill off plants that have not -- sorry
22 -- tissues that have not been transformed.

1 Q. what I'm wondering is at some point you'll
2 start killing off some of the plants that have been
3 transformed, right?

4 MR. FLIBBERT: Objection.

5 A. No, because -- I think I answered the
6 question before.

7 we establish the level in the kill curve I
8 described earlier. So if you use that level there is
9 no way to kill transgenic plants with expressed gene
10 and there will be really no reason to increase the
11 level.

12 BY MR. RUTT:

13 Q. what if there were a bar gene that was a
14 variant of the -- well, strike that.

15 Is the bar gene here that's been used in
16 this experiment wild type?

17 A. I don't know. I don't know.

18 Q. Is there a -- you said you've worked with
19 the bar gene, right?

20 A. Yes.

21 Q. Is there a standard bar gene sequence or
22 does it vary?

1 A. I mean, I've always used the same bar gene
2 in my experiments. So I don't know if other people
3 are using different types of bar genes. But it will
4 be the gene that makes the same enzyme which will
5 have the same enzyme activity. So this is what
6 matters, I think.

7 Q. Right. Suppose somebody made a super
8 strong bar gene that had twice the activity, would
9 that change the kill curve if you were doing
10 experiments on it?

11 MR. FLIBBERT: Objection to form.

12 A. No. I mean, as I mentioned already, the
13 kill curve has established a given level. That level
14 is quite sufficient to distinguish between
15 transformed and non-transformed tissues.

16 So if somebody were to offer me a gene
17 with better activity, I would say I don't need it
18 because I get the result I need.

19 BY MR. RUTT:

20 Q. You've already eliminated all the escapes?

21 A. That's right, yes.

22 Q. Suppose instead somebody made a bad

1 version of the bar gene and had half the activity of
2 the regular bar gene. Then would you need to adjust
3 the kill curve to figure out the new appropriate
4 concentration?

5 MR. FLIBBERT: Objection to form.

6 A. Not really. Again, I think I mentioned
7 it. The level has been established without cutting
8 out a transformation experiment, so I would know what
9 level to use.

10 BY MR. RUTT:

11 Q. What if the bar gene had a variant that
12 was 1 percent, the activity of the bar gene you used
13 in your lab? Then would there have to be a different
14 level of concentration of, say, glufosinate in order
15 to distinguish between transformants and plants that
16 had not been transformed?

17 MR. FLIBBERT: Objection to form.

18 A. Again, I think my answer remains the same.
19 I'm a great believer in kill curves and they never
20 let me down.

21 BY MR. RUTT:

22 Q. Have you ever done experiments with a gene

1 and a variant of that gene that had, say, 1 percent
2 the activity?

3 A. No, I have not.

4 Q. How about have you done experiments with
5 -- experiments like the one here in Zhang, Z-H-A-N-G,
6 with an herbicide-resistant gene and a variant of
7 that herbicide-resistant gene?

8 MR. FLIBBERT: Objection to form.

9 A. I've not done that experiment personally.

10 BY MR. RUTT:

11 Q. Have you ever supervised that sort of an
12 experiment?

13 A. I haven't, but I've read papers and
14 documents about it, and I'm familiar with variants by
15 reviewing the '055 patent.

16 Q. Because that's an example of an AAD-12
17 gene in which it's contemplated there would be
18 variants of that gene, right?

19 A. This is what the patent says.

20 Q. So looking at the text on page 7 -- sorry
21 -- page 41 of Exhibit 2079 it refers to -- oh, the
22 kill curve in the first sentence.

1 Do you see that?

2 A. At the beginning of glufosinate response?

3 Q. That's right.

4 A. I see it, yes.

5 Q. And then in the right column about five
6 lines down the researchers said: "we decided to
7 focus our efforts on selection levels 3.3 or 5.0
8 milligrams per liter."

9 Do you see that?

10 A. I do.

11 Q. And then turning to the next page, page
12 42, there's a subheading titled, Selection Regimes.

13 Do you see that?

14 A. Yes, I do.

15 Q. Just read through that section and onto
16 the next page, if you haven't already done that, and
17 let me know when you're ready to discuss it.

18 A. Okay.

19 Q. So they describe two selection regimes in
20 this section, right?

21 A. They do.

22 Q. And they both lasted for four weeks,

1 right?

2 A. Yes.

3 Q. The second regime they call more
4 stringent.

5 Do you see that?

6 A. They do, yes.

7 Q. why is -- what does that mean?

8 A. It means that the levels of glufosinate in
9 the media were higher.

10 Q. And under the more stringent regime only
11 40 to 50 percent of the explants survived, right?

12 A. Yes.

13 Q. But initially after two weeks under the
14 more stringent regime 70 to 80 percent of the
15 explants had regenerated, right?

16 A. That is correct.

17 Q. So for those 70 to 80 percent of
18 regenerated plants did they have the bar gene?

19 A. I presume they did.

20 Q. why did a subset of that 70 to 80 percent
21 die off in the next two weeks, whereas others
22 survived since it's all the same bar gene?

1 A. Again, this is not uncommon or surprising
2 in plant transformation. We always get that, and
3 this is why I go back to the comments I made earlier.

4 we look out of a transformation
5 experiment. We look at plant populations. And once
6 we identify the winners, shall I say, the plants
7 Number 7 and Number 9 you were referring to before, I
8 would focus on those.

9 The rest do not matter really because this
10 is what -- these are the plants I want to take
11 forward. So, again, it's the same line, the same
12 strategy that I mentioned before. You eliminate the
13 variation during selection.

14 And what matters is the one or two plants
15 that do what you want them to do, and you really
16 don't spend time and resources to figure out why
17 other plants may behave differently.

18 Q. Suppose the researchers had done an even
19 more stringent regime. Would you expect that
20 surviving percentage to drop further from the 40 or
21 50 percent that survived under what's described here?

22 A. I mean, I fail to see the motivation of

1 doing that.

2 Q. It's not something you would ever do in
3 your field, right?

4 MR. FLIBBERT: Objection to form.

5 A. I wouldn't generalize it actually. What I
6 would say for a gene like the PAT gene, I would not
7 do that.

8 BY MR. RUTT:

9 Q. What kind of a gene would you do it for,
10 if any?

11 A. I would do it for metabolic genes or for
12 developmental genes or genes that confer more complex
13 traits which may affect plant metabolism, plant
14 development, plant fertility, things like that.

15 Q. In simple terms for a layperson, why would
16 you make that adjustment to a more stringent regime
17 for those kinds of genes?

18 A. I'm sorry. I may have misunderstood your
19 question. I thought you were talking about selection
20 using the bar gene. And if I did not mishear the
21 question my impression was -- you asked if I would
22 change levels or I would want to change levels of

1 expression of developmental or metabolic change.

2 I'm not referring to selectable markets in
3 any way.

4 Q. I'm sorry. No. My question was: would
5 there ever be scenarios in which a researcher in the
6 field would continue to try to use more and more
7 stringent regimes for selection?

8 A. I mean, if the kill curve gives me the
9 result I need, I would have no motivation or reason
10 to do so.

11 Q. Yeah. It's just not done in your field,
12 right?

13 MR. FLIBBERT: Objection to form.

14 A. Even if the selection works -- and, again,
15 I mean, from my experience with rice and corn and
16 others, I stick to the levels that I know that work.

17 BY MR. RUTT:

18 Q. You call them the winners, right?

19 A. They are the plants I would like to spend
20 time on.

21 Q. You don't care about the losers, the
22 variants that don't perform very well?

1 MR. FLIBBERT: Objection to form.

2 A. I wouldn't call the outcome of a
3 transformation experiment using a selectable marker a
4 variant. They are not variants. They contain the
5 same gene.

6 BY MR. RUTT:

7 Q. Sure. I'm talking about the experiments I
8 believe you indicated you had read about where it's
9 different genes, it's a variant of a given gene.

10 Do you recall that?

11 MR. FLIBBERT: Objection to form.

12 A. Again, I would look at whether any version
13 or any variant, as you mention, encodes the same
14 enzymatic activity or not. And my guidance -- and,
15 actually, this is what I read in the '055 patent.

16 If the enzymatic activity is maintained,
17 then I would have no motivation to change.

18 BY MR. RUTT:

19 Q. Are you ever aware of instances in which
20 the enzymatic activity of a gene variant is not
21 maintained but it's still present?

22 MR. FLIBBERT: Objection.

1 A. These are in general terms?

2 BY MR. RUTT:

3 Q. In general terms.

4 A. Can you please repeat the question?

5 MR. FLIBBERT: Same objection.

6 BY MR. RUTT:

7 Q. Are you aware of an instance where a
8 variant of a gene that expresses something expresses
9 less than the thing that it's a variant of?

10 MR. FLIBBERT: Objection to form.

11 A. I'm not aware. I don't know. I don't
12 know.

13 BY MR. RUTT:

14 Q. In that scenario suppose there were a
15 gene, let's say the AAD-12 gene, and a variant of
16 that gene had 50 percent of the enzymatic activity.
17 In that scenario, would you use the same selection
18 regime concentrations of herbicide to test the
19 variant that has 50 percent of the activity?

20 MR. FLIBBERT: Objection to form.

21 A. Okay. I think I understand your question.
22 So, again, I would go back to basics. And the basics

1 say that if the transgenic plant exhibits the
2 phenotype; in this case, herbicide resistance, then
3 I'll be happy with that.

4 BY MR. RUTT:

5 Q. So suppose the variant does exhibit the
6 phenotype but it only has 50 percent of the level of
7 -- we could call it the wild type. Would you need to
8 adjust the glufosinate levels if that's what you were
9 using as a selectable marker?

10 MR. FLIBBERT: Objection.

11 A. I mean, if we are speaking specifically
12 for the '055 patent, I don't recall reading in the
13 '055 patent that there is any requirement for any
14 level of expression or activity. So in the context
15 of the '055 patent, I don't think it matters.

16 BY MR. RUTT:

17 Q. I'm asking you as a researcher, though, if
18 you wanted to determine whether or not a variant that
19 had far less activity than the AAD-12 gene would you
20 consider using a different level of glufosinate, for
21 example, as a selectable marker in order to determine
22 if it had enzymatic activity?

1 MR. FLIBBERT: Objection to form.

2 A. Since it's a hypothetical question and it
3 doesn't relate to the '055 patent, my answer to the
4 question would be I don't know because I never
5 encountered that situation.

6 BY MR. RUTT:

7 Q. In the papers you read about where there
8 was a wild type gene and a variant of that gene, they
9 were studying the activity levels, you're saying
10 you're not aware of any instance in which the
11 herbicide level of the selectable marker would have
12 had to have been adjusted in order to measure the
13 activity of the variant.

14 Is that what you're saying?

15 A. No, this is not what I'm saying. And,
16 again, I presume this discussion is outside the '055
17 patent. I don't know of such example, so I cannot
18 discuss it.

19 Q. It's not something you considered when you
20 wrote your declaration, right?

21 A. My declaration focuses on the '055 patent.
22 So, again, my starting point was that there is a

1 working gene with activity which detoxified both
2 classes of herbicides. So this was my guidance for
3 writing the report.

4 Q. Yeah. So you didn't consider how a
5 selectable marker regime would or would not have been
6 different for a variant of the AAD-12 gene that had
7 different activity levels, right?

8 MR. FLIBBERT: Objection. Form.

9 A. Again, I go back to what I mentioned
10 earlier. We are talking about a family of AAD-12
11 proteins or variants or whatever which all have the
12 same enzymatic activity.

13 Now, if they have the same enzymatic
14 activity and knowing what I know about very simple
15 traits such as insect tolerance, insect resistance,
16 or herbicide resistance or tolerance, as long as I
17 get -- the protein is the same, the enzymes are the
18 same, then I would not consider that there would be
19 any need to actually do this testing.

20 And, as a matter of fact, when I look at
21 the examples in the '055 patent the same variant
22 worked for multiple species and it worked pretty

1 well. So I don't expect variations of the AAD-12
2 gene that are within the scope of claim 1 of the
3 patent to behave any different.

4 BY MR. RUTT:

5 Q. You assume that variants of the AAD-12
6 gene that are within the scope of claim 1 would have
7 the same or similar activity levels as the sequence
8 that is identified at claim -- in the claims; is that
9 right?

10 A. No. What I refer to is the resulting
11 phenotype which I would measure by seeing if the gene
12 expresses or not.

13 Q. You wouldn't look at how much it
14 expresses; is that right?

15 A. No, because I never saw in the patent or
16 in the claims any specific or -- any specific level
17 of enzymatic activity. So in the context of the
18 documents I reviewed I didn't consider that.

19 Q. And you didn't see in the '055 patent any
20 instances in which they tested a different variant of
21 the AAD-12 gene, right?

22 A. I mean, I don't recall specifically, if

1 you can point me to a place in the patent

2 Q. Let's look at the '055 patent.

3 MR. RUTT: This might be a good time for a
4 break, actually, because this will be -- we'll talk
5 about this for a while. Take a break.

6 THE WITNESS: Okay.

7 (A break was taken.)

8 BY MR. RUTT:

9 Q. welcome back, Dr. Christou.

10 A. Thank you.

11 Q. During the break did you discuss the
12 substance of your testimony with your counsel?

13 A. No, I didn't.

14 Q. So before the break we were talking about
15 whether you had seen any instances in the '055 patent
16 in which the inventors tested a different variant of
17 the AAD-12 gene or different variants of the AAD-12
18 gene.

19 Do you recall that?

20 A. I recall that.

21 Q. Let's look at what variants were tested in
22 the '055 patent. So turn to example 7 of the '055

1 patent. It is at column 48 at the bottom of the
2 page.

3 A. Yes.

4 Q. As far as I can tell this is the first
5 example in the patent of a transformed plant. Does
6 that look correct to you?

7 A. It does.

8 Q. So turn to Table 11 at column 52.

9 A. Okay.

10 Q. These tests results were for the
11 AAD-12(v1) gene, right?

12 A. And I also see (v3) there as well.

13 Q. So I believe that's the AAD-1 (sic) gene?

14 A. I'm sorry. You are absolutely right, yes.

15 Q. So this table shows that they tested the
16 AAD-12(v1) gene, right?

17 A. That is correct.

18 Q. And tables 12 and 13 also indicate that it
19 was the AAD-12(v1) gene that was tested, right?

20 A. That is correct.

21 Q. And the same is true of Table 14, right?

22 A. That is correct.

1 Q. That brings us to example 8. Turn to
2 column 62.

3 A. Yes.

4 Q. At line 36 do you see a section entitled
5 Verification of High 2,4-D Tolerance in T1 corn.

6 Do you see that?

7 A. Yes, I do.

8 Q. And the next line says that "AAD-12(v1)
9 seed were planted."

10 Do you see that?

11 A. I do.

12 Q. And then Table 16 indicates they were
13 testing the AAD-12(v1) gene, right?

14 A. Correct.

15 Q. And Table 17 indicates they were testing
16 the AAD-12(v1) gene, right?

17 A. Correct.

18 Q. And then turn to paragraph 65. Column 65,
19 line 17 says: "This data clearly shows that the
20 AAD-12(v1) conveys high-level tolerance."

21 Do you see that?

22 A. I do.

1 Q. So you understand that the testing of
2 example 8 only involved the AAD-1 -- 12 -- strike
3 that -- the AAD-12(v1) gene?

4 A. I do.

5 Q. So that brings us to example 10 because I
6 don't see any testing in example 9. Do you?

7 A. I'm sorry. Which column are you talking
8 about?

9 Q. Column 65 to 66.

10 A. Thank you. This is --

11 Q. And the question was: Do you see any
12 tolerance testing in example 9?

13 A. I'm sorry. Any tolerance testing,
14 meaning?

15 Q. Testing of tolerance of an herbicide, like
16 2,4-D?

17 A. Tolerance as defined?

18 Q. What does tolerance mean to you?

19 A. Well, I don't. I'm not an herbicide
20 expert.

21 Q. Okay. Well, we can look at the definition
22 of tolerance. Go to column 14, line 56. Column 14,

1 line 55 begins: "As used herein, the term
2 'tolerance' is broader than the term 'resistance,'
3 and includes 'resistance' as defined herein, as well
4 and improved capacity of a particular plant to
5 withstand the various degrees of herbicidally induced
6 injury that typically result in wild-type plants of
7 the same genotype at the same herbicidal dose."

8 Do you see that?

9 A. I do.

10 Q. So does this definition of tolerance help
11 you to understand the question I was asking about
12 example 9, which is whether or not the patent
13 describes any testing of tolerance in example 9?

14 A. well, my understanding is that there is no
15 requirement for a specific level of tolerance. So I
16 didn't consider that.

17 Q. Okay. But I'm asking you a question about
18 example 9, which is at column 65 and 66. I'm asking
19 whether you see any evidence that it was tested for
20 tolerance to any kind of herbicide.

21 A. well, I see tables 18 and tables 19.

22 Q. And they relate to example 8, right?

1 A. Example 9, right? Maybe I'm confused.

2 Are you talking about example 8 or example 9?

3 Q. I'm talking about example 9.

4 A. Example 9 is protein detection from
5 transformed plants via antibody?

6 Q. That's right.

7 A. What was the question again?

8 Q. Do you see any testing results for
9 tolerance to an herbicide in example 9?

10 A. Well, I mean, this does not appear to be
11 the topic of example 9 because it says detection from
12 -- protein detection from transformed plants via
13 antibody.

14 Q. That's my understanding, too. So there is
15 no tolerance testing in example 9, right?

16 A. Again, in the context of the patent, there
17 is no requirement for a specific level. So I don't
18 see the relevance. I'm sorry.

19 Q. Well, whether you see the relevance or
20 not, I'm just asking you the question. Does example
21 9 have any evidence that the inventors tested for
22 tolerance to an herbicide?

1 A. Well, within example 9, no.

2 Q. And on example 10 -- turn to column 68.

3 Do you see Table 20?

4 A. I do.

5 Q. And Table 20 provides for results with
6 respect to the AAD-12(v1) gene, right?

7 A. Yes.

8 Q. And turning the page to Table 21 and 22,
9 23, and 24, all of those relate to example 10 and
10 they all only tested AAD-12(v1) gene, right?

11 A. That is correct.

12 Q. And then looking at example 11 -- turn to
13 column 74. Lines 54 through 58 refer to
14 transformation production of the AAD-12(v1) gene,
15 right?

16 A. That's correct.

17 Q. And at the bottom of column 75 and 76,
18 this is showing the results of testing on the
19 AAD-12(v1) gene, right?

20 A. That is correct.

21 Q. And turning to example 12, go to column
22 81. Lines 39 through 41, do you see that?

1 A. On the left-hand side?

2 Q. That's right. It describes the results
3 for the AAD-12(v1) gene for western blot analysis.
4 Do you see that?

5 A. I do.

6 Q. And in example 13 I don't see any testing
7 results for tolerance, do you?

8 A. I agree.

9 Q. And in example 14 it describes at column
10 84, line 60 the AAD-12(v1) gene.

11 Do you see that?

12 A. I do.

13 Q. And it describes the testing of 2,4-D
14 resistance to the AAD-12(v1) gene, right?

15 A. Yes.

16 Q. And then in example 15 at column 85 you'd
17 agree there's no tolerance test data presented in
18 that example?

19 A. Okay.

20 Q. Do you agree with that?

21 A. I'm sorry. Agree with what?

22 Q. That there's no tolerance test data

1 presented in example 15 for the AAD-12?

2 MR. FLIBBERT: Objection to form.

3 A. I don't see a table, so I would have to
4 read the text.

5 BY MR. RUTT:

6 Q. Go ahead and read it.

7 A. Okay.

8 Q. You'd agree that there are no test data
9 for tolerance presented in example 15?

10 A. That is correct.

11 Q. And in example 16 you see that it's
12 describing the use of herbicides with the AAD-12(v1)
13 gene in the title at column 86, line 25?

14 A. Yes, I do.

15 Q. And the same is true in column 87 for
16 example 17, only the AAD-12(v1) gene is described in
17 example 17, right?

18 A. That is correct.

19 Q. The same is true in column 88 that example
20 18 describes only the AAD-12(v1) gene, right?

21 A. Yes.

22 Q. And turning to column 89, the same is true

1 that example 19 only describes the AAD-12(v1) gene,
2 right?

3 A. Correct.

4 Q. And column 90, example 20 describes only
5 the AAD-12(v1) gene, right?

6 A. Correct.

7 Q. Column 91, example 21 only describes the
8 AAD-12(v1) gene, right?

9 A. Correct.

10 Q. And column 95 in example 22, only the
11 AAD-12(v1) gene is described, right?

12 A. Correct.

13 Q. And column 99, example 23 describes only
14 the AAD-12(v1) gene, right?

15 A. That is correct.

16 Q. And column 100, the example 24 describes
17 only the AAD-12(v1) gene, right?

18 A. That is correct.

19 Q. So all the examples that described testing
20 any kind of tolerance in the '055 patent only
21 described testing in the AAD-12(v1) gene, right?

22 A. That is correct.

1 Q. So I had asked you whether you're aware of
2 any testing of an AAD-12 variant -- strike that.

3 I had asked you whether you're aware of
4 any AAD-12 variants from AAD-12(v1) that were
5 described in the '055 patent. Are you?

6 A. They are not described in the patent. I
7 agree.

8 Q. So returning to the question I was asking
9 about an AAD-12 variant that had 50 percent of the
10 activity of the AAD-12(v1) gene, my question was
11 whether or not you would expect different herbicide
12 levels to be used for the variant as a selectable
13 marker?

14 A. I would not.

15 Q. Why is that?

16 A. Because all the variants no matter -- all
17 the variants encode the same enzyme, on an enzyme
18 with the same enzymatic activity. And knowing what I
19 know for simple traits I wouldn't expect great
20 variation in activity.

21 So I would have no motivation to actually
22 test because I would assume that they will have

1 similar activity.

2 Q. So you would assume you're not going to
3 get a variant that has 50 percent the activity of the
4 AAD-12(v1) gene, right?

5 A. I wouldn't assume that. I mean, I
6 wouldn't know. And, as a matter of fact, I wouldn't
7 care because the specification and the claims of the
8 patent do not call for a specific level of activity.
9 So that would be my reason not to -- not to worry
10 about it.

11 Q. And you wouldn't expect the AAD-12
12 variants within the scope of claim 1 to have an
13 activity level that are 1 percent of the AAD-12(v1)
14 gene, right?

15 MR. FLIBBERT: Objection to form.

16 A. It's the same enzyme. I think it will be
17 very hard for me to imagine that I would see drastic
18 changes in enzymatic activity.

19 BY MR. RUTT:

20 Q. Turn to column 51 of the '055 patent.

21 A. Okay.

22 Q. At line 39 that describes using 300,000

1 seeds that were screened.

2 Do you see that?

3 A. I do.

4 Q. And it says 316 glufosinate resistant
5 plants were identified, right?

6 A. I do.

7 Q. And that equates to a transformation
8 selection frequency of 0.10 percent, right?

9 A. Okay.

10 Q. would you expect that to be in the normal
11 range of selection frequencies for this type of
12 experiment?

13 A. well, this is what the patent says. This
14 is what the sentence says. Since I haven't done the
15 experiment, I have no reason to dispute the result.

16 Q. But that's different from the kinds of
17 rates that you were familiar with in your own work,
18 right?

19 A. In different species, yes, and that's
20 normal as well.

21 Q. This was in the Arabidopsis species,
22 right?

1 A. That is correct.

2 Q. Have you ever worked with Arabidopsis?

3 A. I have.

4 Q. And what kinds of transformation selection
5 rates did you typically see in your work with
6 Arabidopsis?

7 A. I used a very different method and I got
8 frequencies of around 1 to 5 percent, which are
9 pretty good.

10 Q. Certainly better than what's shown in the
11 '055 patent, right?

12 A. I used a different method, so I don't
13 think the two can be compared.

14 Q. Let's turn to Table 11, which is in column
15 52.

16 A. Okay.

17 Q. Take a minute to review the table so we
18 can discuss the results.

19 A. Okay.

20 Q. If you haven't, review column 51, line 35.

21 A. Will you please give me a minute to review
22 this?

1 Q. Sure.

2 A. Okay.

3 Q. So in column 51, lines 49 through 52, it
4 describes how the data are presented as a histogram.

5 Do you see that?

6 A. I do.

7 Q. And it has three categories of plant
8 injury, right?

9 A. Yes, it does.

10 Q. And then the next sentence, it says:
11 "Since each T1 is an independent transformation
12 event, one can expect significant variation of
13 individual T1 responses within a given rate."

14 And that's at column 51, lines 52 to 54,
15 right?

16 A. Yes.

17 Q. Why would one expect significant variation
18 of individual responses because of it being an
19 independent transformation event?

20 A. I presume the question pertains to this
21 specific case of the AAD-12 gene.

22 Q. What does it mean? I don't know what

1 independent transformation event means. I'm curious
2 what it means.

3 A. well, when one carries out a
4 transformation experiment through the selection and
5 regeneration phase one gets a number of different
6 plants out of that experiment. Each one of those
7 plants is unique in terms of its genotype, but it's
8 the same in terms of its phenotype. So this is what
9 the term is.

10 Q. why is each plant unique in terms of its
11 genotype when they all have the same AAD-12(v1)
12 sequence?

13 A. Okay. They are different because
14 different plants would have the transgene located in
15 a different part of their genome. There may be
16 different copies of the genes integrated. So if you
17 sequence the individual results you are going to get
18 differences in the genetic makeup of the plants.

19 That's why they are called independent
20 transformants because they are unique genetic events.
21 Having said that, it means -- I mean, it does not
22 mean that they do not express the same protein.

1 Q. Just different amounts of it, right?

2 A. Different amounts, perhaps, yes.

3 Q. So in Table 11 in the section describing
4 the AAD-12(v1) gene transformants, they have five
5 rows of data, right?

6 A. Yes.

7 Q. And those five rows correspond to
8 different levels of the 2,4-D herbicide treatments,
9 right?

10 A. Yes, from 50 grams active -- 50 grams of
11 active ingredient per hectare up to 3200.

12 Q. And there's also an untreated --

13 A. And there is also an untreated control,
14 yes.

15 Q. So in the 50 gram row, that's the lowest
16 treatment of 2,4-D, right?

17 A. Yes.

18 Q. And there were six -- well, let me ask
19 you: what does the Number 6 mean in that row?

20 A. To me, it means that six of the plants
21 that were tested suffered no or little damage.

22 Q. And then two of the plants suffered severe

1 damage, right?

2 A. That is correct.

3 Q. So they had the same AAD-12(v1) gene, but
4 their genotypes were different, right?

5 A. Correct.

6 Q. And would that be part of what accounts
7 for this difference in injury?

8 A. Yes.

9 Q. What does the column AVE refer to? And
10 I'll point you to column 51, lines 55. So the
11 question is: what does the column AVE refer to?

12 A. It says arrhythmic mean for column 5.

13 Q. What is that describing?

14 A. To be honest, I had the same question. I
15 don't understand.

16 Q. Well, I asked you because I couldn't
17 figure out if the 6 and the 2 in that row refer to
18 percentages or plants.

19 A. I have exactly the same question and I was
20 not able to ascertain that.

21 Q. Okay. And the same question for standard
22 deviation?

1 A. Exactly, yes.

2 Q. Okay. So we don't know. Looking at the
3 AAD-12(v1) section of Table 11, the last row
4 describes the 3200 grams of herbicide.

5 Do you see that?

6 A. That is correct.

7 Q. And it shows that eight plants had little
8 or no injury and zero plants had severe or moderate
9 injury, right?

10 A. That is correct.

11 Q. Does that seem like a discrepancy to you
12 with the 50 gram results?

13 A. Not really because the numbers are so
14 small, so the statistics may show that there is no
15 difference.

16 Q. Because you wouldn't expect for a higher
17 level of herbicide to result in less severe injury,
18 right?

19 A. I'm sorry. Can you please --

20 Q. These results appear strange because the
21 higher level of herbicide at 3200 grams has no severe
22 injury, but the lowest level of herbicide at 50 grams

1 has two with severe injuries.

2 That's unexpected, right?

3 MR. FLIBBERT: Objection to form.

4 A. well, I mean, the difference between
5 testing six plants and testing eight plants may not
6 be statistically significant.

7 So the way that I would interpret this is
8 that most of the plants irrespective of the level are
9 resistant or they don't show any damage if you
10 consider statistical significance in view of the low
11 numbers of plants that I see on the table.

12 BY MR. RUTT:

13 Q. So to figure this out you would need to
14 perform more replicates basically?

15 MR. FLIBBERT: Objection to form.

16 A. I mean, I didn't do the experiment, so I
17 don't know. I mean, the data are there. It says
18 what it says.

19 BY MR. RUTT:

20 Q. In the part of Table 11 that refers to the
21 transformed control -- do you see that?

22 A. Are you referring to the AAD-1 (sic) gene?

1 Q. No, the PAT/Cry1F gene.

2 A. Okay. The BT plant. Yes, I do see that
3 -- sorry -- the Cry1F control.

4 Q. What's the purpose of testing this plant,
5 if you know?

6 A. Well, it's a control. I guess it's to
7 provide a negative control to show that the
8 experiment gives results that are sensible. And I
9 see that the results are sensible because most --
10 actually, with the exception of the lower level of
11 2,4-D, the plants sustained severe injury.

12 Q. What's going on with the lowest level
13 there? Can you explain that to me?

14 A. I cannot.

15 Q. Those plants don't have resistance to
16 2,4-D, right?

17 MR. FLIBBERT: Objection to form.

18 A. I don't know. I presume they don't.

19 BY MR. RUTT:

20 Q. They're the control, right?

21 A. They're the control.

22 Q. So why is it that four of them had no

1 injury and yet at higher concentrations all the
2 plants had injuries?

3 A. I don't know.

4 Q. Could this be explained in terms of an
5 escape?

6 A. No, because an escape is a plant that
7 comes out of a transformation experiment and it is
8 not caught by the selection. So I wouldn't call it
9 an escape.

10 Q. Turn to column 53, line 33. Do you see it
11 says the optimum treatment was 75 grams of 2,4-D?

12 A. The optimum treatment for what?

13 Q. Well, I'll ask you. What is it referring
14 to?

15 A. Yeah, what is it referring to? May I read
16 the preceding paragraph to try to figure it out?

17 Q. Yes.

18 A. Okay. I get it.

19 Q. My reason for the question was that 75
20 grams is higher than 50 grams in the table we were
21 just looking at. So I'm wondering if you think the
22 inventor has increased the minimum dosage to

1 eliminate the strange results they were seeing?

2 MR. FLIBBERT: Objection to form.

3 Mischaracterizes the document.

4 A. I -- I don't know.

5 BY MR. RUTT:

6 Q. Is it possible the control had those four
7 uninjured plants because there just wasn't enough
8 2,4-D being applied to kill off all the plants?

9 A. I don't know. I really don't know.

10 Q. In Table 11, looking at the -- strike
11 that.

12 So turn to column 62. And in line 10 it
13 refers to the copy number of the transgenes.

14 Do you see that?

15 A. Yes, I do.

16 Q. So this is for example 8, right?

17 A. I'm sorry?

18 Q. This is for example 8. Look at column 56,
19 it starts.

20 A. Yes, that's the corn transformation.

21 Q. So the copy number of the transgene varied
22 significantly in this example from one to 15 copies,

1 right?

2 A. It does and it's not unexpected.

3 Q. why is that not unexpected?

4 A. Because this is the nature of the
5 transformation process. It's -- irrespective of
6 which method is used, you're going to end up with
7 transgenic plants which will have different copies of
8 the same transgene. And you see this in every
9 species with every transformation method.

10 Q. Do some species have more copy number of
11 variation than others?

12 A. No.

13 Q. In Table 15 it describes some of the copy
14 numbers.

15 Do you see that?

16 A. I do.

17 Q. And it also has a column that has the
18 acronym ELISA. what is that?

19 A. ELISA.

20 Q. what is ELISA? who is she?

21 A. ELISA refers to a technique called enzyme
22 linked immunosorbent assay, and it's a standard

1 method of detecting proteins.

2 Q. Underneath ELISA in parentheses it says
3 PPM and there's a space, TSP.

4 what does that mean?

5 A. This is a measure of the amount, parts per
6 million of total soluble protein.

7 Q. And what is total soluble protein?

8 A. In this specific case it refers to the way
9 the calculations were done. So in order to carry out
10 an ELISA assay you need to extract total protein --
11 all the protein from the tissue that you may want to
12 determine the amount of the recombinant protein.

13 So what the numbers are telling us is that
14 at least in the first row AAD-12 was represented as
15 146.9 parts per million of the entire soluble protein
16 that was extracted.

17 Q. Okay. That's 1/1,000th or 1/10,000th of
18 total protein was AAD-12?

19 A. Yes, which is quite a lot.

20 Q. It is?

21 A. Yes. For a recombinant protein, that's a
22 huge amount.

1 Q. what does the other protein consist of?

2 A. The other 50,000 proteins that are present
3 in the proteome, P-R-O-T-E-O-M-E.

4 Q. I'm looking at the ELISA column and the
5 copy number column and wondering if there's any kind
6 of relationship between the two?

7 A. Absolutely no relationship, and this is
8 how it should be.

9 Q. why is that?

10 A. Because there is no correlation between
11 transgene expression even at the MRNA level, let
12 alone the protein level in relation to transgene copy
13 number. And this is a solid fact in molecular
14 biology.

15 Q. So I'll point you back to a statement we
16 looked at earlier in the discussion at column 28,
17 Line 41, where it's describing a method of increasing
18 expression of a given gene and indicates copy number.

19 A. Correct.

20 Q. So help me understand how there can be no
21 correlation between the protein of AAD-12 and the
22 copy number and yet the copy number can increase gene

1 expression.

2 A. well, do I have to agree with what I read
3 here?

4 Q. No. I'm asking for your independent
5 expert opinion.

6 A. well, I don't agree with this.

7 Q. Okay. So back to Table 15, what are some
8 of the consequences for gene expression of increasing
9 copy number, if there are any?

10 MR. FLIBBERT: Objection. Beyond the
11 scope.

12 A. I think I already answered this. I don't
13 see any correlation between transgene expression --
14 sorry -- gene copy number and levels of expression.

15 BY MR. RUTT:

16 Q. Can increasing the copy number increase
17 the risk of silencing the gene?

18 MR. FLIBBERT: Objection. Beyond the
19 scope.

20 A. This has got mentioned in some
21 publications. However, there are other publications,
22 including many of my publications which show that

1 that is not the case.

2 BY MR. RUTT:

3 Q. Can increasing the copy number increase
4 the risk of DNA methylation, M-E-T-H-Y-L-A-T-I-O-N?

5 A. No, it cannot because DNA methylation does
6 not have to do with copy number. It has to do with
7 how -- where the gene is located in the genome.

8 Q. Let's look at your book chapter, Exhibit
9 2057, page 127.

10 A. Okay.

11 Q. So in the very first sentence at the top
12 of that page, you write: "It was appreciated that
13 multiple transgene copies could lead to loss of
14 expression (transgene silencing) and that the
15 phenomenon was associated with DNA methylation at the
16 transgenic locus."

17 Do you see that?

18 A. I do see that.

19 Q. So doesn't this mean that copy number
20 could lead to transgene silencing?

21 A. I know better now. So this paper was
22 written in 2002, I believe, or even earlier. Let me

1 see -- 2002. Since then even a lot of my own
2 experiments show that that is not true anymore.

3 Q. And is the same true of the DNA
4 methylation issue?

5 A. Yes.

6 Q. We know now --

7 A. We know a lot more now than we did in 2002
8 and it's not a factor.

9 Q. Did you learn the error of your ways by
10 2005 or 2006?

11 MR. FLIBBERT: Objection to form.

12 A. Well, I mean, the error of my ways, as you
13 put it, is inconsequential because since there is no
14 effect and no impact there was never any reason for
15 concern.

16 BY MR. RUTT:

17 Q. Let's go back to the '055 patent and turn
18 to column 66. This is example 10.

19 A. Okay.

20 Q. And if you look actually at the next page
21 in column 68, there's a Table 20 describing example
22 10 results.

1 Do you see that?

2 A. I do.

3 Q. what does this table show?

4 A. well, the table says tobacco to zero
5 events transformed with a given gene which -- so a
6 given construct which contains the AAD-12(v1) gene
7 plus PAT.

8 And I see here a number of different
9 plants. They have copy numbers for the PAT gene in
10 these plants. They use PCR to show whether the gene
11 is present in the next column. I don't know what the
12 next two columns are. I'm sorry. I have no idea
13 what they mean.

14 And then the last column is entitled
15 Relative Herbicide Tolerance.

16 Q. As a researcher in the field when you look
17 at this table what do you think the inventors were
18 trying to show with this data in 2005/2006 when this
19 was written?

20 A. I mean, I have no idea what they have in
21 their minds, so I'm sorry. I cannot answer the
22 question.

1 Q. But what do you -- as a person of skill in
2 the art, how do you view the meaning of this table,
3 or is it irrelevant?

4 A. Well, it shows some plants that show high
5 levels of tolerance. Others would show variables.
6 Others not tested. Others low. So it just captures,
7 I think, what we were discussing earlier, where if
8 you generated a population of plants you're going to
9 get this diversity of the genotypic level as you can
10 see under the copy number PAT column and also the
11 variation in tolerance which is unrelated with copy
12 number.

13 Q. Why do they show copy number if it's
14 unrelated?

15 A. I have no idea.

16 Q. They should have known better in 2005 or
17 2006, according to you, right?

18 MR. FLIBBERT: Objection to the form.

19 A. I don't know what it was, so I cannot
20 comment.

21 BY MR. RUTT:

22 Q. It could be because they erroneously

1 thought the copy number was related to gene
2 expression, right?

3 MR. FLIBBERT: Objection to form.

4 A. Again, I'm not going to speculate.

5 BY MR. RUTT:

6 Q. But, in your opinion, there's no
7 relationship between the copy number column and the
8 relative herbicide tolerance column, right?

9 A. It's not only my opinion. There is a very
10 substantial portion of my peers in the scientific
11 community who believe likewise.

12 Q. So a person of skill in the art looking at
13 Table 20 in 2005 or 2006 would know that this is
14 irrelevant, right?

15 MR. FLIBBERT: Objection to form.

16 A. I think I answered the question. I mean,
17 I don't know how to -- I mean, I would repeat my same
18 answer.

19 BY MR. RUTT:

20 Q. I mean, you can't identify any relevance
21 to this table, right?

22 MR. FLIBBERT: Objection.

1 A. I mean, all I said is that I cannot
2 understand why column 3 is there.

3 BY MR. RUTT:

4 Q. The copy number column?

5 A. The copy number column. The rest I cannot
6 comment on because I have no idea what the context is
7 or why the inventors did the experiment.

8 Q. And it's not described in the patent, is
9 it?

10 A. I don't know if it's described in the
11 patent or not.

12 Q. I mean, read through column 68 or 67.

13 A. No. If it's not described, it's not
14 described. I don't know.

15 MR. RUTT: Let's take a break.

16 (Whereupon, at 12:36 p.m., a
17 luncheon recess was taken.)

18 - - -

19 A F T E R N O O N S E S S I O N

20 (1:31 p.m.)

21 whereupon,

22 PAUL CHRISTOU, PH.D.

1 was called for continued examination, and having been
2 previously duly sworn was examined and testified
3 further as follows:

4 EXAMINATION BY COUNSEL FOR PETITIONER
5 CONTINUED

6 BY MR. RUTT:

7 Q. welcome back, Mr. Christou -- Dr. Christou
8 -- apologies.

9 A. Thank you.

10 Q. During the break did you discuss the
11 substance of your testimony --

12 A. No.

13 Q. -- with your counsel?

14 A. No, we did not.

15 MR. FLIBBERT: Make sure he finishes the
16 question before you answer.

17 THE WITNESS: Sorry.

18 BY MR. RUTT:

19 Q. Turn to Exhibit 2057, which is your book.
20 Turn to page 114.

21 A. Okay.

22 Q. The section heading on that page says

1 Contransfer -- one word -- of Vector Backbone
2 Sequences by Agrobacterium.

3 Do you see that?

4 A. I do.

5 Q. what does that mean?

6 A. It means that on occasion during the T-DNA
7 transfer process not only the transgenes get
8 transferred into the genome of the host plant, but
9 also segments of sequences of the vector are used to
10 carry out the transformation.

11 Q. And why did you include a section in your
12 book on that topic?

13 A. Because there was a perception in the
14 literature that agrobacterium only transfers whatever
15 is located in between the T-DNA borders of the TI
16 plasmid into the host genome, and because of that the
17 resulting transformants were clean as the terminology
18 was at the time.

19 Q. I see here in the first sentence you say:
20 "Agrobacterium was once believed to result in,
21 quote, clean transformation, end quote."

22 Do you see that?

1 A. Yes.

2 Q. That's what you're referring to?

3 A. Yes. That's what I referred to.

4 Q. So you said previously some of your
5 opinions in this 2002 book changed.

6 Do you recall that?

7 A. Yes.

8 Q. Did your opinion about this topic of
9 vector backbone sequences change in the intervening
10 years?

11 A. It's not that it changed, but vectors were
12 improved to such an extent that after the period we
13 wrote this paper it was no longer an issue. It used
14 to be an issue at the dawn of technology and then
15 that was rectified.

16 Q. Was it still an issue in 2002?

17 A. In 2002 it was still an issue, yes.

18 Q. Was it still an issue in 2005 or 2006?

19 A. To be honest, I don't recall when the
20 developments in the agrobacterium vector construction
21 happened, because in my work I used mostly direct DNA
22 transfer. Sorry. I cannot answer that because I

1 don't know.

2 Q. But at least as of 2002 it was still an
3 issue?

4 A. Yes.

5 Q. In the third paragraph of that section on
6 page 114 of Exhibit 2057, the first sentence begins:
7 "Further investigations have shown that vector
8 sequence transfer is probably a very common event."

9 Do you see that?

10 A. I do, and I see the references. May I
11 check the dates just to make sure I put this in
12 context, please?

13 Q. Sure.

14 A. So the three references date back to 1996
15 and 1997. So this is the period that the statement
16 refers to.

17 Q. So I'll show you an exhibit --

18 A. Do I need to keep this open?

19 Q. Yeah, keep it open.

20 A. All right.

21 Q. I like to move around.

22 A. Okay. That's fine.

1 MR. RUTT: I'll show you a new exhibit
2 marked 1044.

3 MR. FLIBBERT: And I'll just object for
4 the record on the ground of authentication, hearsay,
5 relevance, and outside the scope of the declaration.

6 BY MR. RUTT:

7 Q. Dr. Christou, do you recognize this
8 document?

9 A. I do.

10 Q. What is this document at a high level?

11 A. It's a review article we wrote, meaning
12 members of my team and some collaborators, discussing
13 mechanisms that control expression of transgenes in
14 plants.

15 Q. And when was this published?

16 A. It was published in 2006.

17 Q. And you are the author listed for purposes
18 of correspondence, right?

19 A. That is correct.

20 Q. Are you the most senior author on the list
21 of coauthors?

22 A. Yes, I am.

1 Q. So turn in that exhibit to page 187.

2 A. Okay.

3 Q. You've prepared a very nice figure. What
4 does it show in figure 1 if you're able to summarize
5 all of those lines and labels?

6 A. This has been such a long time. I need to
7 look at it for a little bit.

8 Q. Take your time and let me know when you're
9 ready to discuss it.

10 A. Thank you. Okay.

11 Q. What are you attempting to show in figure
12 1?

13 A. Well, I need to put it in context, and
14 this was the knowledge at the time of writing. I
15 must say that a lot of the things that are shown in
16 this figure are no longer as important as they used
17 to be because of changes in technology and our
18 ability to do transformation much better.

19 So at the time of writing --

20 Q. Just to be clear, what was the time of
21 writing?

22 A. I believe that was 2006.

1 Q. July, August 2006?

2 A. That is correct. I'm sorry. I lost my
3 train of thought.

4 Q. I'm sorry to interrupt you. I'm curious
5 what portions of this figure 1 you would consider
6 being no longer accurate since 2006?

7 A. I wouldn't say they are inaccurate. I
8 think I pretty much answered that by saying that the
9 technology has developed to such an extent that many
10 of these aspects are no longer an issue.

11 And, essentially, one of the major factors
12 that is no longer an issue is because we now
13 appreciate that even the early tools we had back in
14 2005, that time period, could eliminate a lot of
15 these problems at the time of selection.

16 Q. So, for example, I see near the bottom of
17 the chart there's a bubble kind of right in the
18 center of the page that says: "Minimal vector
19 sequence or substantial vector sequence."

20 Do you see that?

21 A. I do.

22 Q. What is that referring to?

1 A. To be honest, I don't recall without
2 getting a bit of understanding of the context.

3 Q. Take your time and look at the article.

4 A. Okay. Thank you. I guess it refers to
5 different scenarios one may encounter in terms of how
6 transgenic loci are organized after integration.

7 Q. And this is one of the mechanisms that at
8 the time, 2006, you believed controlled the
9 expression of introduced genes in crop plants?

10 A. It's not a mechanism. It's a factor
11 amongst all the other factors that show here. But,
12 again, as I reiterated, a simple selection process
13 would eliminate all of these issues.

14 Q. And we now know that --

15 A. No. We were doing it inadvertently from
16 the very beginning, but we never really recognized
17 the fact.

18 Q. Staying with that figure 1, there's a box
19 just underneath the vector sequence material that
20 says position effects.

21 Do you see that?

22 A. I do.

1 Q. What are position effects?

2 A. I mean, as we discussed earlier, position
3 effects refer to where in the genome a transgene
4 integrates after integration.

5 Q. And why are they relevant to our
6 understanding of the mechanisms that control the
7 expression of introduced transgenes in crop plants?

8 A. Yes. They are relevant because depending
9 on where in the genome of a host plant a gene will
10 integrate, that environment will impact transgene
11 expression.

12 Q. And since writing this in 2006, has the
13 field changed its view of importance or the
14 significance of position effects?

15 A. I think it does. And, again, the change
16 is retroactive because you can eliminate and negate
17 completely position effects through selection, which
18 was practiced from the very beginning of plant
19 biotechnology.

20 Q. And I see on here there is a bubble that
21 refers to head-to-head or head-to-tail.

22 Do you see that?

1 A. Yes, I do.

2 Q. This was something we had discussed
3 earlier as having a potential impact on transgene
4 expression, right?

5 A. That is correct.

6 Q. And has the field's thinking since 2006 on
7 the relevance of this factor changed at all?

8 A. Well, in principle, it hasn't changed, but
9 it's no longer a factor again because of the
10 selection process which identifies expressing plants.

11 Q. At the bottom of the chart there's a
12 triangle that says silencing.

13 Do you see that?

14 A. I do.

15 Q. What does that mean as you use it here in
16 figure 1?

17 A. It means that under certain conditions,
18 which are listed above the triangle, transgenes may
19 be silenced.

20 Q. And has the field's thinking on the
21 conditions that lead to gene silencing changed since
22 you wrote this article in 2006?

1 A. Again, as I mentioned earlier, the
2 principle remains the same. However, in practice it
3 can only be eliminated at the selection step.

4 Q. Because we know more now, right?

5 A. No, because the selection inadvertently
6 takes care of this.

7 Q. To the right of figure 1 there's a line of
8 text near the top of the page that's in bold font.

9 Do you see that?

10 A. I do.

11 Q. It says, why Single Copy Transgene Loci
12 Might Not Always be Desirable or Ideal in Transgenic
13 Crops. And you have it as a question; is that right?

14 A. Correct.

15 Q. So is this a statement that reflected your
16 thinking in 2006?

17 A. Not necessarily. It may or may not still
18 be the case now. But, again, the issue of copy
19 number -- I think we discussed already -- there's no
20 impact in practical terms because during selection
21 this -- what I'm trying to say in response to this
22 line of questioning is that the selection process

1 takes care of all these factors that may influence
2 transgene expression because it selects for plants
3 that express irrespectively of copy number, position
4 effects, transgenic locus, architecture, and the
5 like.

6 So this is why I don't worry too much now
7 about this. So this article was written to delve
8 into the detailed factors that affect transgene
9 expression. But, again, as I mentioned already, all
10 these factors can be negated at the selection step.

11 Q. What does figure 2 show?

12 A. Okay. It shows different levels of
13 expression, the mRNA level in figure B. Figure 8
14 shows the transgene integration patterns at the DNA
15 level. And figure C if I can find -- where is C? I
16 don't see it in the caption. But the writing is too
17 small, I'm afraid, for me to read what it says on the
18 scale for figure C.

19 Q. I think it might say relative CDNA. Would
20 that make sense?

21 A. No, it would not make sense.

22 Q. It must not be that.

1 A. I'm sorry. I cannot read it.

2 Q. That's okay. In the description of figure
3 2a it refers to the integration of SAMDC in one, two,
4 or three sites.

5 Do you see that?

6 A. I do.

7 Q. What does that mean?

8 A. The figure shows the DNA patterns of the
9 transgene integrated in these three independent lines
10 and it shows that each one of them is different.

11 Q. The caption refers to "variable levels of
12 expression in specific transgenic rice lineages."

13 Do you see that?

14 A. I do.

15 Q. Is there anything on here that indicates
16 those variable levels of expression in figure 2?

17 A. Part B shows different levels of MRNA
18 expression, indeed. And, again, I wish I could read
19 what figure C is. I'm sorry. I cannot read it.

20 Q. That's okay.

21 A. Definitely there is variation in
22 expression at least at the MRNA level, and I would

1 also expect because of that at the protein level as
2 well, again, which is normal amongst the population
3 of transgenic plants.

4 Q. So in figure 2b does darker blobs mean
5 heavier expression? greater expression?

6 A. At the MRNA level, yes.

7 Q. So for the first construct, Number 98,
8 there was one integration site, right?

9 A. I mean, it's not a construct. It's a
10 transgenic plant line. The construct is the same in
11 all of them, in all the lines.

12 Q. So for the transgenic plant Number 98
13 there was one locus where it was integrated?

14 A. Indeed, one locus.

15 Q. And for the second one, Number 105, there
16 were two loci?

17 A. Correct.

18 Q. And for the third one, Number 106, there
19 were three loci?

20 A. Correct.

21 Q. And in figure 2b it looks like Number 105,
22 the second one, which had two loci had at the least

1 amount of mRNA expression.

2 Am I reading that right?

3 A. You are.

4 Q. And then it looks like a tie between
5 Number 98 and Number 106 in terms of the amount of
6 mRNA expressed; is that right?

7 A. Which is great, yes.

8 Q. Why is that great?

9 A. Because out of three plants two of them
10 express at high level, which is pretty good.

11 Q. Did it have -- did those expression levels
12 have anything to do with what's shown in figure 2a?

13 A. Absolutely nothing.

14 Q. And did you know that at the time you
15 wrote the article or is that something that --

16 A. No. I knew it at the time that I wrote
17 the article.

18 Q. So why did you show figure 2a and figure
19 2b beside each other if there's no relationship?

20 A. Because a lot of peers in the scientific
21 community believed that it was.

22 Q. So this was to help enlighten them that

1 they're wrong, basically?

2 MR. FLIBBERT: Objection.

3 A. This was to state the facts as coming out
4 of this experiment, yes.

5 BY MR. RUTT:

6 Q. Just looking at what's shown in figure 2,
7 are you able to identify the reasons why the second
8 plant, Number 105, had less mRNA expression than the
9 other two plants?

10 A. I would have no motivation to investigate
11 that. I would, again, focus on the other plants. I
12 would leave that plant behind.

13 Q. But I'm asking you now: Do you have any
14 idea why?

15 A. No. And to be honest, I don't care. I
16 didn't care then, and I don't care now.

17 Q. Let's go back to the '055 patent. Turn to
18 Table 25, which is at column 75 and 76 and continuing
19 to column 77 and 78.

20 Do you see that?

21 A. I do.

22 Q. And this refers to data about the soybeans

1 that were transformed in example 11, right?

2 A. That is correct.

3 Q. So go ahead and study that table and let
4 me know when you're ready to discuss it.

5 A. Okay. Okay.

6 Q. So the bottom of the table shows
7 experiments on gene WT. Does that mean wild type?

8 A. It does.

9 Q. These are plants without the AAD-12 gene
10 in them, right?

11 A. Correct.

12 Q. And it appears there were two different
13 amounts of the herbicide 2,4-D applied to the wild
14 type plants, right?

15 A. Yes, I see that.

16 Q. There was 560 grams and 1120 grams, right?

17 A. Correct.

18 Q. There's a column third from the right that
19 says: "Percent injury two days after treatment."

20 Do you see that?

21 A. I do.

22 Q. In the plants that received the lower dose

1 of the herbicide between 50 percent and 70 percent
2 were showing injury at the second day after
3 treatment, right?

4 A. Correct.

5 Q. Does that mean that 50 percent to 30
6 percent were not showing any injury at that time?

7 A. I don't know, meaning I can only infer
8 what I read here. And I read that between 50 and 70
9 percent of the plants sustained injury. So it looks
10 as though that most of the plants at this lower level
11 -- at this level sustained injury early on, which if
12 we look a little bit later, the injury is a lot more
13 severe and it's 100 percent at the higher levels.

14 Q. Why doesn't the injury to the plants that
15 had the lower amount of herbicide reach 100 percent?

16 A. I presume 100 percent means that the
17 plants were dead and I think 60 and 80 percent is an
18 attempt to quantify death, perhaps. So I am sure for
19 all practical intents and purposes these plants were
20 not looking great anyway.

21 Q. And did these experiments -- is it fair to
22 say the goal is to apply a level of herbicide that

1 clearly distinguishes between plants that have the
2 herbicide resistant genes and those that do not?

3 A. well, I'm not an herbicide scientist, so I
4 don't know the intent. All I can do is interpret
5 what I see in front of me, I'm afraid.

6 Q. Looking at the rows that refer to plants
7 with the AAD-12 gene, the bottom six rows have levels
8 of herbicide applied to them.

9 Do you see those rows?

10 A. I do.

11 Q. And I'm looking now at the amounts of the
12 ELISA scores for those six rows. And I see that they
13 are very different from each other; is that right?

14 A. That is correct and it's quite normal.
15 I'm not surprised.

16 Q. what do you think could be some of the
17 factors that caused those differences in ELISA
18 amounts?

19 MR. FLIBBERT: Objection. Form.

20 A. Again, we're going to (inaudible), which
21 is not my area of expertise. Again, all I can do is
22 comment on what I see.

1 BY MR. RUTT:

2 Q. Looking at the rows of the AAD-12 plants,
3 do you see that two of them have 5 percent injury on
4 day two?

5 A. I do.

6 Q. And they had fairly significant
7 differences in the ELISA measure of expressed AAD-12
8 protein, right?

9 A. Correct.

10 Q. The first one, which is D-5-2 event has
11 less than 1 nanogram of AAD-12 per milliliter, right?

12 A. Yes.

13 Q. And the second one, which is event number
14 D-4-17 has 286 nanograms per milliliter of AAD-12,
15 right?

16 A. Yes.

17 Q. They both produced the same amount of
18 percent injury, right?

19 A. This is what I see.

20 Q. Even though the one had a protein
21 concentration that's 4- or 500 times the amount of
22 the other, right?

1 A. Correct.

2 Q. Now, they did have a difference in the
3 herbicide concentration applied, right?

4 A. Yes, they did.

5 Q. The one has two times the amount of
6 herbicide as the other, right?

7 A. Yes.

8 Q. But it has 500 or 400 times the amount of
9 protein, right?

10 A. Yes.

11 Q. So why wouldn't the injury and the one
12 with so much more protein have been less?

13 MR. FLIBBERT: Objection. Beyond the
14 scope.

15 A. I mean, the way I would interpret this --
16 and, again, I'm not an herbicide scientist -- is that
17 the damage most likely has nothing to do with the
18 herbicide.

19 It could be that there was a component in
20 the mix that was used for the experiment perhaps as
21 surfactant because something else that appeared to
22 influence these plants.

1 I mean, my conclusion is that this injury
2 here most likely is not related to the herbicide for
3 the simple reason that it disappears over time. At
4 least this is the conclusion I would draw.

5 BY MR. RUTT:

6 Q. Do you think that number where it's 1
7 nanogram per milliliter or less than 1 nanogram per
8 milliliter is correct?

9 A. You mean the 0.58?

10 Q. Yeah.

11 A. I have no idea.

12 Q. It's very low, and yet it protected the
13 plant from 95 percent of the injuries, right?

14 A. Well, if this is -- the number is correct,
15 what it's telling me is that we are dealing with a
16 very, very good gene.

17 Q. It must be extremely effective, right?

18 A. It looks like it.

19 Q. But then why did it get injured at all at
20 the row that had 286 nanograms of the gene?

21 A. I think I answered this before, and I
22 think the answer has to be the same. In looking at

1 the data I'm not convinced that the injury is because
2 of the herbicide. It could be because of something
3 completely different.

4 Q. Is all of the protein that's measured in
5 that ELISA number an active protein or is some of it
6 inactive?

7 A. It's active ELISA by definition measures,
8 function, and protein.

9 Q. Let's turn to column 47 of the patent.
10 Example 6 describes the in vitro activity of AAD-12
11 on various substrates.

12 Do you see that?

13 A. Correct.

14 Q. In your work as a plant transformation
15 scientist, do you work with in vitro samples of
16 plants or in vitro testing of plants? This may be a
17 better way to ask the question.

18 A. well, it depends on the testing. what
19 kind of testing? Can you please clarify?

20 Q. Sure. Let's say herbicides, substrates.

21 A. I don't work with herbicides.

22 Q. So let's go to column 100. This is

1 example 24, which is testing AAD-12(v1) as an in
2 vitro dicot selectable marker.

3 Do you see that?

4 A. I do.

5 Q. Around lines 34 through 40 it describes
6 using 2,4-D.

7 Do you see that?

8 A. Yes, I do.

9 Q. What does it mean when it says: "Use of
10 2,4-D in vitro for cell-level selection is
11 complicated by the fact 2,4-D is used almost
12 ubiquitously as a plant growth regulator in the plant
13 tissue culture systems"?

14 A. 2,4-D is an auxin, A-U-X-I-N. And auxin
15 is one of the two major classes of plant hormones
16 that are used in in vitro culture to induce and
17 proliferate cell differentiation to produce callus.

18 Q. And so what's so complicated about that?

19 A. In what context?

20 Q. Well, the patent says: Use is complicated
21 by the fact that 2,4-D is used as a plant growth
22 regulator.

1 what's the patent referring to?

2 A. Okay. I mean, 2,4-D is an herbicide so it
3 would kill a plant. But at a certain amount 2,4-D is
4 an important hormone which is used to induce and
5 proliferate tissue cultures. So this is a
6 complication.

7 Q. Turn to column 101, which is describing
8 more about example 24.

9 Do you see that?

10 A. I do.

11 Q. At the very bottom of the column at lines
12 59 through about 62, do you see it says: "There was
13 little growth observed in all the selection
14 concentrations of 2,4-D tested above 10 milligrams
15 per liter"?

16 A. I see that.

17 Q. And then at the top of page 102, the first
18 three lines say: "It appears that 10 milligrams per
19 liter 2,4-D is the sublethal."

20 Do you see that?

21 A. I do.

22 Q. And above that concentration 2,4-D appears

1 to be lethal to the non-transformed cells?

2 A. I see that, yes.

3 Q. So it appears the inventors were doing
4 dose response testing here, correct?

5 A. well, I don't see a dose response. I only
6 see 10 milligrams per liter in the sections which --
7 in the section that we just went through. So I
8 cannot tell if they tested any other levels.

9 Q. Sure. So read the first two paragraphs of
10 column 101.

11 A. Okay. Fine. Yes. I see that it was a
12 kill curve experiment.

13 Q. And you've worked with kill curve
14 experiments, right?

15 A. I have.

16 Q. But you haven't worked with herbicides?

17 A. As herbicides. In the field I've never
18 worked with them, no. I've worked with molecules
19 that are being used as herbicides, but I've never
20 done any herbicide testing or tolerance tests or
21 anything in the field or in the greenhouse to assess
22 herbicides' tolerance or resistance. All I've done

1 was to use them in selectable markets.

2 Q. So I'll ask you some questions about
3 example 24. And if you -- you're not able to answer
4 because you're not working with herbicides, that's
5 fine. Just let me know.

6 A. Okay.

7 Q. In lines 11 through 16, they refer to
8 different levels of herbicide used.

9 Do you see that?

10 A. I do.

11 Q. It's one, two, three, five, eight, ten,
12 12, 15, 18, 20, 40, 60, 80 -- it looks like a typo.
13 Probably should have been 100 -- 110, 120 milligrams
14 per liter 2,4-D.

15 Do you see that?

16 A. I do.

17 Q. So they tested all these concentrations;
18 is that right?

19 A. Yes.

20 Q. And then at the bottom in lines 59 through
21 62, they say: "Several fast-growing colonies were
22 found in 2, 5, and 8 milligrams per liter of 2,4-D

1 concentration."

2 Do you see that?

3 A. I do.

4 Q. And then further down the page at line 64,
5 they also sampled colonies from the 12, 15, 18, and
6 20 milligrams per liter 2,4-D.

7 Do you see that?

8 A. Yes, I do.

9 Q. But for those colonies there were only a
10 few of them, right?

11 A. This is what it says here, yes.

12 Q. And is that because even for the AAD-12
13 transformed plants there was a concentration past
14 which the callus would not grow?

15 A. I'm sorry. Can you please repeat the
16 question?

17 Q. Sure. So is the idea that once the
18 herbicide level exceeds 10 milligrams per liter
19 there's approaching a threshold where the callus just
20 does not grow much more?

21 A. Are we referring to wild type callus --

22 Q. No.

1 A. -- or --

2 Q. No, the transformed type.

3 A. well, that would indicate a threshold, I
4 guess, yes.

5 Q. Okay. I'm looking at column 102, lines 15
6 through 17 now.

7 A. Oh, okay. So I was looking at column 101
8 -- I'm sorry. Yes. So can you please repeat the
9 question? I'm sorry to make you repeat it.

10 Q. That's okay. I'm wondering if -- in
11 column 102, line 15 it indicates there's a 20
12 milligram per liter 2,4-D concentration that is a
13 threshold beyond which the callus did not grow; is
14 that right?

15 A. That is correct.

16 Q. And that's the callus of the transformed
17 plants; is that right?

18 A. Yes.

19 Q. They don't report the -- whether there
20 were fast-growing colonies at 1 milligram per liter,
21 even though they tested that, right?

22 A. well, if they don't, they don't.

1 Q. They report 2, 5, and 8 as the
2 fast-growing colonies, right, at line 61 of column
3 101?

4 A. Yes. That is correct.

5 Q. And there was -- in line 59 they report
6 there was little growth observed and all selection
7 concentrations of 2,4-D tested above 10 milligrams
8 per liter, right?

9 A. I'm not sure if -- I mean, does this refer
10 to the transgenic calli or the wild type? I'm not
11 sure about this. May I please read this --

12 Q. Please read it all.

13 A. Thank you. Okay. Now, it's better.

14 Q. Okay. So were these referring to
15 transformed cells?

16 A. Yes, they were.

17 Q. So cells that contained the AAD-12 gene?

18 A. Yes.

19 Q. Okay. So above 10 milligrams per liter
20 the transformed cells showed very little growth?

21 A. That is correct.

22 Q. And they showed fast-growing colonies at

1 several concentrations below 10 milligrams per liter,
2 correct?

3 A. That is correct. And I think this is the
4 determining factor in terms of the selection compared
5 to the wild type. So it looks as though the
6 selective agent is very efficient and you don't
7 really need very high levels to get selection. This
8 is how I will interpret the result.

9 Q. Do you know whether the colonies would be
10 fast-growing at concentrations above 10 milligrams
11 per liter if there were an AAD-12 variant that was
12 twice as active as the wild type?

13 A. No.

14 MR. FLIBBERT: Objection.

15 BY MR. RUTT:

16 Q. You don't know?

17 A. I mean, the selection as described works.
18 So, again, I don't know if it's important or not.

19 Q. Okay. But I'm asking you -- whether it's
20 important or not, I'm asking you if in the
21 hypothetical where there's an AAD-12 variant that
22 performs with twice the activity of the variant that

1 was tested here, would it grow fast at concentrations
2 of 2,4-D that were higher than the concentrations
3 used here?

4 A. I don't know.

5 MR. FLIBBERT: Objection.

6 A. Sorry. I don't know.

7 BY MR. RUTT:

8 Q. That's not something you considered when
9 you wrote your declaration, right?

10 A. well, I think the point I considered is
11 that the variants of AAD-12 would all have similar
12 activity. So I don't see how a variant would have
13 higher activity and fall within the scope of claim 1
14 in terms of the conserved motive of the region and
15 the sequence identity of up to 85 percent.

16 So my expectation would be that it would
17 be very hard to find out -- I would be very surprised
18 if a variant would exhibit those properties. So that
19 would be my expectation.

20 Q. And that would be the same whether the
21 variant is exhibiting activity high above the variant
22 tested here or far below the variant; is that right?

1 A. No. My answer was and still is that my
2 expectation, it would be that -- I would be very
3 surprised if a variant would exhibit those
4 properties.

5 So I mean, from a technical point of view,
6 knowing what I know and reading the '055 patent, I
7 find it very difficult to expect the variant to
8 behave the way you describe.

9 MR. RUTT: Okay. Let's take a break.

10 (A break was taken.)

11 BY MR. RUTT:

12 Q. welcome back, Dr. Christou. During the
13 break did you discuss the substance of your testimony
14 with your counsel?

15 A. No, I did not.

16 Q. I want to look back again at Exhibit 2079.

17 A. Yes.

18 Q. I have a question about Table 2, which is
19 on page 41. Read the caption to yourself and
20 familiarize yourself with it because I'd like some
21 help understanding the columns.

22 A. Okay.

1 Q. So in Table 2 what does the column labeled
2 N refer to?

3 A. Well, according to the caption, it's the
4 number of explants that were put into culture in the
5 transformation process.

6 Q. And then the next column says total number
7 in G.H. what does that mean?

8 A. The caption says it indicates greenhouse.

9 Q. And then the next column says: "I.E. in
10 G.H. indicates the number of independent lines
11 (plants arising from independent explants)
12 represented."

13 Do you see that?

14 A. Yes, but I don't understand it.

15 Q. Okay.

16 A. I really don't.

17 Q. I don't either. I was curious if you
18 could shed light on it?

19 A. Absolutely no idea. I find this table
20 very confusing, to be honest.

21 Q. I do, too.

22 A. Yes.

1 Q. They appear to be calculating percent GUS
2 plus efficiency by looking at the column labeled GUS
3 plus and dividing it by the column labeled N.

4 Does that look right?

5 A. This is what it says. But, again, I have
6 absolutely no idea what it means or what the
7 calculations mean.

8 Q. Okay. Turn in this exhibit to figure 3 on
9 page 43.

10 A. Okay.

11 Q. So this is showing the different fragments
12 of different soybean plants that have been
13 transformed, right -- fragments of DNA -- strike
14 that.

15 This is showing fragments of DNA from
16 transformed soybean plants, right?

17 A. This is what it shows, yes.

18 Q. And the fact that there are different
19 patterns for the different plants is consistent with
20 there being a different insertion location of the
21 transgene; is that right?

22 MR. FLIBBERT: Objection to the form of

1 the question.

2 A. Can you please rephrase the question so I
3 understand it?

4 BY MR. RUTT:

5 Q. Yeah. So I'm just wondering if -- you
6 know what -- strike that. That's fine.

7 Turn in your declaration to paragraph 144.

8 A. Okay.

9 Q. And in the second sentence of that
10 paragraph, you say: "The '055 patent claim language
11 does not specify any particular level of protein
12 expression or herbicide degradation activity."

13 Do you see that?

14 A. I do.

15 Q. So if an AAD-12 variant has herbicide
16 degradation activity that is so low that it cannot be
17 detected by looking at the leaves of a plant, that
18 would still be herbicide activity within the scope of
19 the claims, right?

20 MR. FLIBBERT: Objection to the form of
21 the question, and it calls for a legal conclusion.

22 A. I'm trying to understand your question.

1 Can you please try to rephrase?

2 BY MR. RUTT:

3 Q. I can rephrase. Suppose there's an AAD-12
4 variant that has 1 percent of the activity of the
5 variant that was tested in this patent and as a
6 result it doesn't show any difference in the -- from
7 the wild type plants when 2,4-D is applied.

8 Did you get that hypothetical?

9 A. I'm afraid not.

10 Q. Okay. Suppose you have two plants, one is
11 wild type, one has an AAD-12 variant that has
12 extremely low activity, so low that when 2,4-D is
13 applied there's no difference in the effect on the
14 leaves between the variant and the wild type.

15 Do you understand the hypothetical?

16 A. I think I do.

17 Q. would that 2,4-D plant that has very
18 little activity still be herbicide activity as
19 required by the claims?

20 MR. FLIBBERT: Objection to the form of
21 the question. Objection. Calls for a legal
22 conclusion.

1 A. I believe if the plant has activity this
2 is what the claim requires, to the best of my
3 understanding. So if it has activity, I would say
4 it's within the scope.

5 BY MR. RUTT:

6 Q. The claims don't require --

7 A. The claims do not require a specific
8 level.

9 Q. Let's look at claim 1 of the '055 patent.
10 It's in column 125.

11 A. Okay.

12 Q. Claim 1 does not require that the activity
13 produce a result in the visual appearance of the
14 leaves of a plant, does it?

15 A. There is absolutely no mention of
16 activity. So I don't see any indication of levels of
17 activity other than the requirement to possess the
18 ability to create these two classes of herbicides and
19 also being consistent with the two stipulations of
20 the claim.

21 Q. So in Line 4, you see it says, "said
22 activity enzymatically degrades"?

1 Do you see that?

2 A. Yes, I do.

3 Q. That activity does not need to produce a
4 result in the appearance of a plant leaf in order for
5 it to be activity within the scope of the claim,
6 right?

7 MR. FLIBBERT: Objection to the form.
8 Objection, calls for a legal conclusion.

9 A. Can you please rephrase?

10 BY MR. RUTT:

11 Q. Sure. You said -- I believe you said the
12 claims don't require activity. So the first thing I
13 wanted to clarify is that --

14 A. No. If I said that, I misspoke.

15 Q. Okay.

16 A. What I meant was that the claims do not
17 require a particular level of activity.

18 Q. Such as?

19 A. A level of activity that would result in
20 visually damaging the -- strike that.

21 Such as a level of activity that would
22 protect the plant from damage to its leaves as a

1 result of herbicides being applied, right?

2 MR. FLIBBERT: Objection to the form.

3 Objection, calls for a legal conclusion.

4 A. I mean, I'm a little bit confused. All I
5 can say is that I don't see any requirement in the
6 claim or the specifications of the '055 patent that
7 stipulates a particular level of activity.

8 BY MR. RUTT:

9 Q. Do you see how -- turn in your declaration
10 to paragraph 63 but keep the patent open.

11 A. Paragraph 63?

12 Q. 63.

13 A. Okay.

14 Q. In the -- this paragraph you describe some
15 of the claims of the patent that depend from claim 1.

16 Do you see that?

17 A. I do.

18 Q. And the third bullet describes claim 4.

19 Do you see that?

20 A. I do.

21 Q. And you write that "a transgenic plant
22 comprising a plurality of the plant cells of claim

1 1."

2 Do you see that?

3 A. I do.

4 Q. So you'd agree that claim 1 is directed to
5 a transgenic plant cell, right?

6 A. Within the specifications stipulated in
7 claim 1, yes.

8 Q. And you'd agree that claim 4 is directed
9 to a transgenic plant, correct?

10 A. Again, referring to -- derived from the
11 claim of -- sorry -- derived from the plant cell
12 claimed in claim 1.

13 Q. Claim 1 does not require a transgenic
14 plant, right, just the transgenic plant cell?

15 A. The claim describes a transgenic plant
16 cell.

17 Q. And claim 4 describes the transgenic
18 plant, right?

19 A. Which is derived from the transgenic plant
20 cell referred to in claim 1.

21 Q. Right. So claim 1 doesn't require a plant
22 at all, just a plant cell, right?

1 A. I believe I answered that. The claim
2 talks about a plant cell.

3 Q. Claim 4 refers to the "expression of said
4 polynucleotide," P-O-L-Y-N-U-C-L-E-O-T-I-D-E,
5 "renders said plant tolerant to an herbicide."

6 Do you see that?

7 A. I do.

8 Q. In claim 9, it refers to rendering said
9 plant resistant to both herbicides.

10 Do you see that?

11 A. I do.

12 Q. What's your understanding of the
13 difference between tolerance and resistance, if
14 there's any?

15 MR. FLIBBERT: Objection. Beyond the
16 scope.

17 A. It's very hard for me, at least, to
18 distinguish a difference between the two.

19 BY MR. RUTT:

20 Q. Was it something you considered when you
21 were providing your opinion in this declaration, the
22 difference between resistance and tolerance?

1 A. I mean, what I consider was whether a
2 plant would express the phenotype of being -- and I
3 used -- I guess I used the words interchangeably,
4 resistance or tolerance.

5 So what I consider in forming my opinion
6 was whether the plant would exhibit a tolerant or
7 resistant phenotype, and I equated the two because
8 I'm not an expert in herbicides. So I don't know if
9 that's right or wrong, but this is what I used.

10 Q. And we talked about how claim 1 requires
11 an activity, right? Do you see that?

12 A. Yes, I do.

13 Q. What's your understanding of the
14 difference between an activity and tolerance, if
15 there's any difference?

16 MR. FLIBBERT: Objection to the form.

17 A. Can you please repeat it?

18 BY MR. RUTT:

19 Q. What's the difference in your mind between
20 the meaning of the word "activity," as used in claim
21 1, and the meaning of the word "tolerant," as used in
22 claim 4, if you think there's any difference at all?

1 MR. FLIBBERT: Objection to the form.
2 Objection, calls for a legal conclusion.

3 A. I guess the way I would answer your
4 question would be that if the plant cell of claim 1
5 exhibits any kind of activity that would fall -- that
6 would be within the scope of the claim.

7 BY MR. RUTT:

8 Q. And you'd understand tolerance or tolerant
9 to be more than mere activity; is that right?

10 MR. FLIBBERT: Objection to form.
11 Objection, beyond the scope.

12 A. I don't know.

13 MR. RUTT: No further questions.

14 EXAMINATION BY COUNSEL FOR PATENT OWNER

15 BY MR. FLIBBERT:

16 Q. I have just a few questions.

17 Dr. Christou, do you recall being walked
18 by counsel through the examples of the '055 patent to
19 confirm that the examples were performed using the
20 AAD-12(v1) variant?

21 A. Yes, I do.

22 Q. You were then asked the question along the

1 lines of whether the patent describes AAD-12 variants
2 other than the (v1) variant.

3 Do you recall that?

4 A. I do.

5 Q. Do you have the '055 patent?

6 A. I do.

7 Q. Could you please turn to column 19?

8 A. Okay.

9 Q. Beginning at line 51, do you see the
10 discussion in that paragraph concerning variant
11 proteins and equivalent proteins?

12 A. I do.

13 Q. That discussion continues onto column 20;
14 is that correct?

15 A. Correct.

16 Q. Could you please turn to column 21?

17 A. Okay.

18 Q. Do you see the paragraph that begins at
19 line 41?

20 A. Yes.

21 Q. Could you please just briefly read that
22 paragraph to yourself?

1 A. Yes, I did.

2 Q. Do you see a discussion there of variant
3 or equivalent proteins?

4 MR. RUTT: Objection. Leading.

5 A. No. I see a lot of discussion, yes.

6 BY MR. FLIBBERT:

7 Q. And does that discussion continue through
8 onto column 22?

9 A. It does.

10 Q. Did counsel walk you through any of the
11 disclosures in columns 19 through 21 of the '055
12 patent relating to variant and equivalent proteins?

13 A. No, he did not.

14 Q. Dr. Christou, regarding the enzymology
15 aspects of the '055 patent, have you been asked to
16 give any opinions about the sufficiency of the
17 specifications disclosure of AAD-12 variants?

18 A. No, I have not.

19 MR. FLIBBERT: I have no further
20 questions.

21 THE REPORTER: Reading and signing, Mr.
22 Flibbert?

1 MR. FLIBBERT: Yes, please.

2 THE REPORTER: And would you like a copy?

3 MR. FLIBBERT: Yes, please.

4 THE REPORTER: And eTran, hardcopy, or
5 both?

6 MR. FLIBBERT: The same as last time.

7 THE REPORTER: And would you like a rough?

8 MR. FLIBBERT: Yes, please.

9 THE REPORTER: Mr. Rutt, you have a
10 standing order?

11 MR. RUTT: I think so.

12 THE REPORTER: Thank you.

13 (Signature having not been waived, the
14 deposition of Paul Christou, Ph.D. was concluded at
15 3:17 p.m.)

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C E R T I F I C A T E

I do hereby certify that I am a Notary Public in good standing, that the aforesaid testimony was taken before me, pursuant to notice, at the time and place indicated; that said deponent was by me duly sworn to tell the truth, the whole truth, and nothing but the truth; that the testimony of said deponent was correctly recorded in machine shorthand by me and thereafter transcribed under my supervision with computer-aided transcription; that the deposition is a true and correct record of the testimony given by the witness; and that I am neither of counsel nor kin to any party in said action, nor interested in the outcome thereof.

WITNESS my hand and official seal this
_____ day of _____, 2024.

Notary Public

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Please read your deposition over carefully and make any necessary corrections. You should state the reason in the appropriate space on the errata sheet for any corrections that are made.

After doing so, please sign the errata sheet and date it.

You are signing same subject to the changes you have noted on the errata sheet, which will be attached to your deposition.

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Exhibits Exhibit 1001 4:18 19:8,9 Exhibit 1044 5:11 Exhibit 2012 4:11 9:14 Exhibit 2057 4:15 10:16 13:6,9 22:2 98:8,9 104:19 107:6 Exhibit 2076 4:20 30:20 31:20 Exhibit 2079 5:7 50:2 59:21 136:16 \$ \$20,000 12:13 \$525 12:6 0 0.10 83:8 0.58 125:9 055 9:4 10:11 19:12 21:4 59:15 65:15 67:12, 13,15 68:3,16,21 69:21 70:19 71:2,15, 22 80:20 81:5 82:20 84:11 99:17 119:17 136:6 139:10 141:9 143:6 147:18 148:5 149:11,15 1 1 51:2,5,6,14 52:2,14, 19 54:10 58:12 59:1 70:2,6 82:12,13 84:8 109:4,12 110:5 111:18 113:16 114:7 123:11 125:6,7 132:20 135:13 140:4 141:9,12 143:15	144:1,4,7,12,13,20, 21 146:10,21 147:4 1.5 38:21 39:19 40:6,16 1/1,000th 95:17 1/10,000th 95:17 10 54:12 74:5 77:2,9 93:12 99:18,22 128:14,18 129:6 131:18 133:7,19 134:1,10 100 80:16 121:13,15,16 126:22 130:13 1001 19:9 101 128:7 129:10 132:7 133:3 102 128:17 132:5,11 1043 31:17 42:6 1044 108:2 105 117:15,21 119:8 106 117:18 118:5 11 72:8 77:12 84:14 87:3 89:3 90:20 93:10 120:1 130:7 110 130:13 111 13:20 112 22:3 1120 120:16 114 104:20 107:6 12 8:13 43:19 45:7 47:6 49:15 72:18 74:2 77:21 130:12 131:5 120 130:13	125 141:10 127 98:9 12:36 103:16 13 43:12 72:18 78:6 14 37:13 72:21 74:22 78:9 144 139:7 146.9 95:15 15 7:5 78:16 79:1,9 93:22 94:13 97:7 130:12 131:5 132:5, 11 16 73:12 79:11 130:7 17 73:15,19 79:16,17 132:6 18 75:21 79:20 130:12 131:5 187 109:1 19 75:21 80:1 148:7 149:11 1993 31:5 1996 107:14 1997 107:15 1:31 103:20 1a 38:6,10 1g 38:7,10 2 2 41:9,12,13 42:7,10 43:9 48:5 52:2,14 54:10 88:17 115:11 116:16 119:6 130:22	133:1 136:18 137:1 2,4-D 73:5 74:16 78:13 87:8,16 91:11,16 92:11 93:8 120:13 127:6,10,11,14,21 128:2,3,14,19,22 130:14,22 131:6 132:12 133:7 135:2 140:7,12,17 2.5 38:21 39:19 40:6,16 20 12:1,11 77:3,5 80:4 99:21 102:13 130:12 131:6 132:11 148:13 20-fold 47:13 2002 13:2 98:22 99:1,7 106:5,16,17 107:2 2005 17:2 99:10 101:16 102:13 106:18 110:14 2005/2006 100:18 2006 99:10 101:17 102:13 106:18 108:16 109:22 110:1,6 111:8 112:12 113:6, 22 114:16 2012 9:14 2057 10:16 13:6,9 22:2 98:9 104:19 107:6 2062 10:17 2065 10:22 2076 30:20 31:20 2079 50:2,6 59:21 136:16 21 77:8 80:7 148:16 149:11 22 77:8 80:10 149:8 23
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