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D E C I S I O N
of 8 November 2000

Case Number: T 1054/97 - 3.3.4

Application Number: 91108536.3

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IPC: C12N 15/32

Language of the proceedings: EN

Title of invention:
Modifying plants by genetic engineering to combat or control insects

Patentee:
Aventis CropScience N.V.

Opponent:
Agrigenetics LP
Novartis AG Patent and Trademark Dept.

Headword:
Insects control/AVENTIS

Relevant legal provisions:
EPC Art. 123(2)(3), 84, 54, 56

Keyword:
"Novelty - main request - no"
"Inventive step - first and second auxiliary request - no, third auxiliary request - yes"

Decisions cited:
G 0001/92, T 0989/93

Catchword:
-

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Case Number: T 1054/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 5 November 2000

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 18 August 1997 concerning maintenance of European patent No. 0 451 878 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
C. Holtz

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division issued on 18 August 1997 whereby the European patent No. 0 451 878 with the title "Modifying plants by genetic engineering to combat or control insects", with 10 claims for all Designated Contracting States was revoked pursuant to Article 102 EPC.

II. In response to a communication indicating the Board's provisional, non binding opinion, the Appellants (Patentees) on 24 February 2000 filed a new main request. Claims 1, 2, 3 and 9 read as follows:

"1. A chimaeric gene which comprises:
a promoter region derived from a gene which is naturally expressed in a plant cell, such as a Pnos, PTR2, Pssu pea, Pssu 301, or P35S; and
a 3' untranslated region, including a polyadenylation site, of a gene which is naturally expressed in a plant cell, such as 3'ocs, 3't7, 3'nos, or 3'SSu301, and
a coding sequence encoding only part of the Bt2 protein of Fig.13, said protein part extending from nucleotide position 141 to a nucleotide position between nucleotide positions 1961 and 2314 in Figure 13."

"2. DNA comprising the DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605."

"3. A protein or an insecticidally active, truncated protein encoded by the DNA of claim 1 or 2, with the proviso that said protein is not the protein illustrated in Chart A of EP-A-0 206 613, from amino acid position 1 to amino acid position 610, or from amino acid position 1 to amino acid position 608, or

from amino acid position 1 to amino acid position 608 with Val-Lys-His added on to the C-terminus."

"9. A method for combatting Lepidoptera comprising applying to the Lepidoptera the protein or truncated protein of claim 3 or 4."

III. Oral proceedings were held on 8 and 9 November 2000. During these proceedings, three auxiliary requests were filed.

Claim 2 of auxiliary request I read as follows:

"2. The DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605."

Claim 2 of auxiliary request II read as follows:

"2. A protein or an insecticidally active, truncated protein encoded by the DNA of claim 1 or 2 *(sic)*, with the proviso that said protein is not the protein illustrated in Chart A of EP-A-0 206 613, from amino acid position 1 to amino acid position 610, or from amino acid position 1 to amino acid position 608, or from amino acid position 1 to amino acid position 608 with Val-Lys-His added on to the C-terminus."

Auxiliary request III contains as the sole claim, claim 1 of the main request.

IV. The following documents are referred to in the present decision:

(1) Kronstad, J. et al., Journal of Bacteriology, Vol. 154, No. 1, pages 419 to 428, 1983,

(5) Schnepf, H.E. and Whiteley, H., The Journal of Biological Chemistry, Vol. 280, No. 10, pages 6273 to 6280, 1985,

(7) Shibano, Y. et al., Gene, Vol. 34, pages 243 to 251, 1985,

(9) Wabiko, H. et al., Applied and Environnemental Microbiology, Vol. 49, No. 3, pages 706 to 708, 1985,

(11) Klier, A. et al., The EMBO Journal, Vol. 1, No. 7, pages 791 to 799, 1982,

(22) Lereclus, D. et al., The EMBO Journal, Vol. 3, No. 11, pages 2561 to 2567, 1984,

(23) Adang et al., Gene, Vol. 36, pages 289 to 300, 1985,

(26) Vaeck, M. et al., Nature, Vol. 328, pages 1 to 5, 1987,

(27) Lereclus, D. et al., Mol.Gen.Genet., Vol. 186, pages 391 to 398, 1982,

V. The arguments by both parties in writing and during oral proceedings insofar as they are relevant to the present decision can be summarized as follows:

Main request:

Rule 88 EPC; claim 1
Respondents

- There was no basis in the application as filed for the insecticidal part of the protein extending to nucleotide position 2314. The argument by the Appellants that the skilled person would understand from the description as filed that the position 2308 (originally filed claim 2) was, in fact, position 2314 because this latter position was the position of the Kpn I restriction site at the end of the smaller fragment with full toxicity (page 16, lines 55 and 56) was not valid because the skilled person would have no reason to look in the description to interpret the claim and, thus to gain knowledge about an obvious mistake (Rule 88 EPC).

Appellants

- In claim 2 as filed, the insecticidal protein encoded by the chimaeric gene was characterized as extending from nucleotide position 141 (ATG) to any nucleotide position from 1961 to 2308 whereas in claim 1 now under consideration, it was characterized as extending from position 141 to any nucleotide position from 1961 to 2314. The skilled person reading page 16, lines 55 to 56 would readily recognize that the smallest DNA fragment encoding a protein with full insecticidal activity ended at the Kpn I site. The Kpn I site was characterized in Figure 13 as being at position 2314. Thus, it would be immediately obvious that the reference to position 2308 in claim 2 as filed (also identified as position 2167 on page 16, line 49, not taking into account the nucleotides preceding the ATG) was an error as no Kpn I site was found in position 2308. A

correction of the end position from nucleotide 2308 to nucleotide 2314 was thus obvious and allowable under Rule 88 EPC.

Article 84 EPC: clarity
Respondents

- The reference in claim 1 to the nucleotide position 2314 as end point of the coding sequence rendered the claim unclear as the nucleotide 2314 was in the middle of a codon.

- Claim 3 was unclear as it made reference to the DNA of claim 1 or 2 whereas neither of these claims disclosed any DNA.

Appellants

- The skilled person would have no difficulty in identifying the insecticidal coding sequence comprised between nucleotides 141 and 2314 in the light of Figure 13. Claim 1 was clear.

- The wording in claim 3 :"A protein or...truncated protein encoded by the DNA of claim 1 or 2" was clear even if said claims 1 and 2 did not explicitly refer to DNA because they referred to a **coding sequence** encoding ...the Bt2 protein, which would be understood as being DNA.

Article 123(2)(3) EPC
Respondents
claim 1

- The wording "a coding sequence encoding only part of the Bt2 protein" in claim 1 was intended to cover Bt2 coding sequences fused to any other amino acid sequences as was made clear in claim 3 dependent on claim 1 which comprised fused Bt2 coding sequences (some of which where disclaimed in the proviso of the claim). Thus, claim 1 comprised any fused constructs containing any Bt2 encoding DNA of intermediate length and degenerate coding sequence. Yet, the application only disclosed the specific NTPII marker gene as fused marker and Bt2 DNA with substantial homology to the one specifically disclosed as the insecticidal DNA to be expressed. It could not serve as a basis for such a wide claim.

- The claim covered RNA genes which were not comprised in the granted claims and, thus, was not allowable under Article 123(3) EPC.

Claim 9:

The application as filed did not disclose a method for combatting Lepidoptera which involved applying the B.t.berliner 1715 insecticidal protein to plants and, thus, the requirements of Article 123(2) EPC were not fulfiled.

Appellants
Claim 1

- The subject-matter of this claim found support in the application as filed on page 3, lines 27 to 29 as well as lines 38 to 48, page 7, line 32, claims 6 and 7, where chimaeric genes were

disclosed in a generic manner without any limitation as to the size or to a specific sequence of the insecticidal gene in the chimaera.

- Claim 1 was not directed to RNA genes as the claimed construct could not be assembled starting from RNA. The scope of the claim had not been extended (Article 123(3)EPC).

Claim 9:

The disclosure in the application as filed on page 27, line 22, page 29, lines 4 to 7 and page 45, line 5 made it implicit that the insecticidal protein or part thereof could be used in a method to combat Lepidoptera.

Article 54 EPC; claim 2
Appellants

The 42 Mdal natural plasmid of Bacillus thurigiensis Berliner 1715, (B.t.berliner 1715) disclosed in document (27) did not affect the novelty of claim 2 for the following reasons:

(i) the 42 Mdal plasmid could not easily be separated from the other plasmids within the strain.

(ii) the gene encoding the insecticidal protein (insecticidal gene) which was described in the state of the art prior to the priority date (document (11)) was not located on the 42 Mdal plasmid but on a plasmid of a higher molecular weight as shown in Figure 4B, lane 2 of this document. And, besides, the probe used to locate

the insecticidal gene hybridized to more than one B.t. insecticidal gene, which cast doubt on the origin of the isolated DNA fragment containing the insecticidal gene.

(iii) even if it was accepted that the probe hybridized to a 14 Kb BamHI subfragment of the 42 Mdal plasmid, there existed no means of identifying which open-reading frame encoded the insecticidal gene in this fragment.

(iv) there were two insecticidal genes in B.t.berliner 1715.

(v) the experiments filed by the Respondents to show the identity of the insecticidal gene carried by the natural 42 Mdal plasmid of B.t.berliner 1715 to the insecticidal gene described in the patent in suit were neither legally nor technically valid. They had been filed a long time after the filing date of the patent in suit. Furthermore, the Respondents had not compared the sequence of the claimed insecticidal gene with that of the 42 Mdal plasmid insecticidal gene, but with that of another insecticidal gene which did not have the same restriction map as the 42 Mdal plasmid insecticidal gene.

Respondents

Document (27) which disclosed the 42 Mdal plasmid of B.t.berliner 1715 was novelty-destroying to the subject-matter of claim 2:

- At the filing date, this plasmid could be

isolated without difficulty. Indeed, the very fact that it was stated on page 392 of document (27) that "the 39 and 42 Mdal plasmids were not always distinctly resolved" implied that they could be resolved. It contained only one insecticidal gene, the location and sequence of which could have been identified.

- The Respondents had shown beyond doubt that the insecticidal gene carried by the natural 42 Mdal plasmid and cloned in plasmid pBT42-1 (document (11)) was the same as the insecticidal gene disclosed in the patent in suit by showing that their sequences were the same. The argument by the Appellants that the toxin gene which had been compared to the toxin gene of the patent in suit was not the toxin gene of the 42 Mdal plasmid because it did not have the same restriction map as this latter gene, was irrelevant, the observed differences being simply due to mapping errors.

Auxiliary request I

Article 123(2) EPC
Appellants
Claim 2

A basis in the application as filed for the DNA fragment with the sequence given in Figure 13 in isolated form could be found on page 14, lines 53 to 54.

Article 56 EPC
Appellants

The closest prior art was document (1) which described the use of a B.t.kurstaki DNA probe to localize the insecticidal gene in 32 different strains of Bacillus thurigiensis. It also disclosed that the DNA encoding the B.t.kurstaki insecticidal protein had been cloned and its 5' end had been partially sequenced.

Starting from this closest prior art, the problem to be solved could be defined as providing the insecticidal gene of Bacillus thurigiensis.

In 1983, it had not been established whether the insecticidal genes were all different. The skilled person aware of the teaching of document (1) would have no incentive to look for more genes.

If the experiment was tried nonetheless, it could not have been reasonably expected that the claimed B.thurigiensis gene would be isolated as the teachings of document (1) were that the B.t.kurstaki DNA hybridized to the DNA from many Bacillus thurigiensis strains and, besides, B.t.berliner 1715 would certainly not have been chosen as starting material since it was known that the strain contained no less than 17 plasmids. Finally, had the skilled person started cloning the toxin gene from B.t.berliner 1715 on the basis of the information given in document (11), he/she would not have been able to identify the open-reading frame of the insecticidal gene once cloned.

Respondents

The closest prior art document was document (11) which disclosed that an insecticidal gene was present on the 42 Mdal plasmid of B.t.berliner 1715, which plasmid was

available to the public (document (27)). Document (11) also taught which probe to use to retrieve the gene from the 42 Mdal plasmid and specified that it was located on a 14 Kb BamHI fragment. The person skilled in the art would have found it a matter of routine firstly to reclone the 14Kb BamHI fragment and secondly, to tailor it down to the claimed 3 Kb fragment containing the toxin gene.

Auxiliary request II

Article 56 EPC ; claim 2
Appellants

The filing date of the patent in suit was the priority date for the claimed insecticidally active truncated proteins. The state of the art comprised documents (5),(23),(7) and (9). In document (5), it was shown that the shortest protein of B.t.kurstaki HD-1 Dipel with insecticidal activity had a molecular weight of 78 Kd. In B.t.kurstaki HD-73, it was a 68 Kd truncated protein which remained insecticidally active (document (23)). In B.t.berliner 1715, toxicity was still retained by a construct expressing a 65 Kd and a 100 Kd protein (document (9)). In view of all these different results, the skilled person would have been unable to predict the size of the B.t.berliner 1715 truncated insecticidal proteins which would retain toxic activity. The claimed insecticidal proteins were inventive.

Respondents
Claim 2 to insecticidally active truncated proteins

The closest prior art document was document (9) which

disclosed that only part of the protoxin gene of B.t.berliner 1715 was necessary for insecticidal activity and also that the toxicity could be due to a 65 Kdal truncated peptide. Thus, it could be fully expected that truncated proteins as claimed, having a molecular weight between 68 Kdal and 100 Kdal would be toxic.

Auxiliary request III

Article 56 EPC
Appellants

The closest prior art was document (9). This document disclosed that the expression in E.coli of deleted fragments of the B.t.berliner 1715 insecticidal gene encoding an insecticidal protein of at least 100 Kd led to truncated proteins which were very toxic to tobacco hornworm larvae. On the contrary, the expression in E.coli of a shorter DNA fragment did not lead to the synthesis of a stable protein (pH1, Figure 2 and page 708, first para.)

The problem to be solved was to provide constructs which led to efficient insecticidal resistance in plant cells.

The solution was the isolation of constructs carrying deleted fragments of the insecticidal gene encoding an insecticidal protein of less than 100 Kd. This solution was in direct contradiction with the results obtained when E.coli was used as a host for expression. They could not have been expected and, thus, the subject-matter of claim 1 was inventive.

Document (5) disclosed that the minimum length of the B.t.kurstaki HD-1 Dipel DNA fragment expressing a truncated protein with insecticidal activity was 645 codons i.e the size of the deleted fragments of B.t.berliner 1715 used in plant cells. Yet, there was no reason why this result would have suggested that the B.t.berliner 1715 insecticidal gene could also be so shortened and still encode a stable truncated protein in view of the instability observed in document (9).

The Respondents' argument that the insecticidal effect in plant cells had only been documented with one specific chimaera and thus could not serve to justify acknowledging inventive step over the whole scope of the claim was not relevant in the absence of any proof on their part that other chimaera would not be toxic and the phenomenon had been proven with more than one construct (Ex.10.8, page 30 of the patent in suit together with document (26)).

Respondents

In document (9), a construct, pH1, was disclosed which comprised a deleted insecticidal gene, the expression of which did not lead to the synthesis of a stable, truncated, insecticidal protein. The deleted insecticidal gene was much shorter than the ones used for expressing insecticidal proteins in plants. Thus, its failure to encode a stable, truncated, insecticidal protein did not imply that the same failure would occur with the constructs used in plant cells. Document (5), on the contrary, disclosed that a deleted insecticidal gene from B.t.kurstaki HD1-Dipel of the same size as the deleted, insecticidal genes claimed to be used in plants led to the expression of an insecticidally

active truncated protein. Taking into account that the insecticidal genes of B.t.berliner 1715 and B.t.kurstaki were highly homologous, the skilled person would have expected that B.t.berliner 1715 DNA fragments of the same size as that of the B.t.kurstaki deleted DNA fragments would share the same properties i.e. lead to the expression of an insecticidally active truncated protein. The claimed constructs, thus, lacked inventive step.

The insecticidal effect in plant cells was only observed with one construct where the deleted insecticidal gene had been fused to a marker gene. This was insufficient for inventive step to be acknowledged over the whole scope of the claim.

VII. The Appellants requested that the decision under appeal be set aside and that the patent be maintained on the basis of either of the main request filed on 24 February 2000, the first or second auxiliary request filed on 8 November 2000 or the third auxiliary request filed on 9 November 2000.

The Appellants further requested that an obvious mistake in claim 1 of all requests be corrected under Rule 88 EPC.

The Respondents requested that the appeal be dismissed.

Reasons for the Decision

Main request:

Rule 88 EPC

1. In the originally filed claim 2, the DNA encoding the insecticidally active part of the protein is defined as that shown in Figure 13 extending to a nucleotide position between nucleotide positions 1961 and 2308. The Appellants argued that in light of the description, page 16, lines 55 to 56 (published version of the application) and of Figure 13, the skilled person would immediately recognize that the DNA encoding the insecticidally active part of the protein extended to a nucleotide position between nucleotide positions 1961 and 2314 rather than from 1961 to 2308 and that, therefore, the correction of 2308 to 2314 introduced in claim 1 of the main request now to be considered was allowable under Rule 88 EPC, which states that the requested correction "must be obvious in the sense that it is immediately evident that nothing else would have been intended than what is offered as the correction".

2. On page 16, lines 55 to 56 of the published version of the application as filed, it is taught that the smallest restriction fragment encoding the entire active toxic unit ends at the Kpn I restriction site. Figure 13 which provides the sequence of a cloned fragment comprising the insecticidal gene shows that the Kpn I site is at position 2314. In the Board's judgement, the skilled person would, thus, have no difficulty in understanding that the DNA fragment encoding the toxic part of the protein extends to position 2314 rather than to position 2308. The argument by the Respondents that the skilled person reading the originally filed claim 2 would have no reason to suspect that it contained a mistake and, thus, would not turn to the description for

interpretation is not convincing. Indeed, the skilled person interested in the claimed invention would read all that relates to it including, of course, the patent specification as a whole and, thus, would become aware that the mentioning of nucleotide position 2308 in originally filed claim 2 was a mistake.

3. It is concluded that the correction of the expression in claim 1 "between nucleotide positions 1961 and 2308" to "between nucleotide positions 1961 and 2314" is allowable under Rule 88 EPC.

Article 123(2) EPC

Claim 1

4. In the application as filed page 3, lines 27 to 30 and 40 to 45, the chimaeric construct is defined as comprising a promoter derived from a gene which is naturally expressed in a plant cell, any DNA fragment encoding an insecticidally active protein and any marker gene fused to it. On page 7, lines 43 to 45, it is also disclosed that the chimaeric gene may include a 3' non-translated region. The specific promoter regions, 3' non-translated regions and the coding sequences referred to in the claim are mentioned on page 10, lines 13 to 17, Table 7 and original claim 2 respectively. Thus, the subject-matter of claim 1 finds support in the application as originally filed.

Claim 9

5. The application as filed, page 4, line 18 discloses insecticidal compositions. In the Board's judgment the method of claim 9 (see section II, supra) is, thus,

implicitly but unambiguously disclosed.

6. The requirements of Article 123(2)EPC are fulfilled.

Article 123(3) EPC

7. The argument that claim 1 covered RNA genes which were not included in the granted claims is not convincing because this would require that the claimed chimaeric construct is assembled from DNA (the plant promoter) and RNA (the RNA "gene"), which is an obvious impossibility. The requirements of Article 123(3) EPC are fulfilled.

Article 84 EPC

8. The Respondents argued that claim 1 was unclear because it referred to a protein sequence encoded by the DNA ending at position 2314 whereas nucleotide 2314 is not the third nucleotide of a triplet.

9. Figure 13 and the description on page 16, lines 55 and 56 show that the position 2314 defines the cutting site of the Kpn I enzyme and not the last base in a codon. Accordingly, the skilled person would understand that the last amino-acid of the insecticidal protein mentioned in claim 1 was that encoded by the last triplet comprised within the restriction fragment.

10. It was also argued that claim 3 was unclear because it made reference to a DNA of claim 1 or 2 whereas the word DNA did not appear in any of these claims. Claim 1 and claim 2 (by being dependent on claim 1) make reference to a coding sequence. Thus, claim 3 is not ambiguous as it is self-evident for the skilled person

to equate the terms "coding sequence" and "DNA" in the given context.

11. The requirements of Article 84 EPC are fulfilled.

Novelty

12. According to both parties, claims 2,4,7,8, and 10 enjoy priority from the priority date and the other claims enjoy priority from the filing date. The Board agrees to this point.

13. The Respondents argued that claim 2 relating to **a DNA comprising** the DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605 lacked novelty over the 42 Mdal plasmid described in document (27). This document provides a study of the plasmids contained, in particular, in B.t.berliner 1715, i.e in the strain, the insecticidal gene referred to by its sequence in claim 2, is isolated from. The existence of the 42 Mdal plasmid in B.t.berliner 1715 is shown on page 393, Table 1.

14. The Enlarged Board of Appeal decision G 1/92 (OJ 1993, 277) stipulates that "the chemical composition of a product is state of the art when the product as such is available to the public and can be analysed and reproduced by the skilled person irrespective of whether or not particular reasons can be identified for analysing the composition. The same principle applies mutatis mutandis to any other product."

15. In the Board's judgment, taking into account the gist of this decision leads to the conclusion that document (27) is indeed novelty destroying to the subject-matter

of claim 2 because, as will be shown in points 16 and 17 below, it was common general knowledge at the priority date that the 42 Mdal plasmid could be obtained in isolated form and contained an insecticidal gene which could have been identified and shown by standard techniques to comprise the DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605.

16. Three years before the priority date of 18 January 1985, the 42 Mdal plasmid had already been used as a DNA probe (document (11), page 793, 2nd para.) which, of course, implies that it had been obtained in isolated form. Its preparation is mentioned in the Materials and Methods section of document (11). This document also shows that the 42 Mdal plasmid carries the insecticidal gene on a 14 Kb BamHI fragment (page 795). At the priority date, the skilled person would have had difficulty neither in isolating the 42 Mdal plasmid from B.t.berliner 1715 nor in obtaining 14 Kb BamHI fragment containing the insecticidal gene, on the basis of this common general knowledge.

17. In the Board's opinion, he/she would also have been able to analyse this fragment in terms of its sequence and to locate the insecticidal gene therein by virtue of its homology to the B.t.kurstaki insecticidal gene, on the basis of the then prevailing general common knowledge relative to DNA sequencing and comparison of sequences. Indirect evidence to support this opinion comes from the fact that a DNA fragment containing the insecticidal gene of B.t. kurstaki HD-1-Dipel had already been cloned and its coding region localized as early as 1981 and 1983 respectively (document (5), page 6273, right-hand column).

18. The Appellants challenged the soundness of the experimental results pre-dating the priority date. They argued on the basis of the observation in document (27) that the 42 Mdal plasmid could not **always** be separated from the 39 Mdal plasmid which was also present in the B.t.berliner strain, that the 42 Mdal plasmid could not be isolated. In the Board's judgment, this observation, on the contrary, provides evidence that it could be obtained in isolated form. They also put forward the argument on the basis of a comparison in document (11) of the distances of migration on a gel of the B.t.berliner 1715 plasmid hybridising to the cloned insecticidal gene and of the 47 Mdal plasmid of B.kurstaki (Figure 4B, lines 2 and 3) that the B.t.berliner 1715 plasmid naturally carrying the insecticidal gene was not the 42 Mdal plasmid but a plasmid of a molecular weight higher than 47 Mdal. In Figure 4B, however, the 47 Mdal plasmid appears to migrate differently depending on where it was initially loaded on the gel (lines 3 and 5). The reliability of the data in Figure 4B may, thus, be put into question. Accordingly, the Board does not see these data as being a reason to doubt the otherwise clear teaching of document (11) on page 791 (left-hand column) and page 795 (beginning of the first paragraph) that the insecticidal gene is on the 42 Mdal plasmid.

19. Furthermore, the Appellants argued in respect of the Respondents' evidence that the sequence of the insecticidal gene present in the 14 Kb BamHI fragment of the 42 Mdal plasmid was identical to that of the DNA of Figure 13 (DNA sequencing report filed with their submissions received on 11 July 1997) that this evidence was not acceptable on a legal point of view, having been submitted too late, as well as on a

technical point of view, the sequenced fragment being, in their opinion, different from the 14 Kb fragment known in the art before the priority date (document (11)).

20. It should firstly be remarked that comparative evidence with the claimed sequence could only be produced after the claimed sequence was available. Thus, it cannot be held against the Respondents that they carried out their analysis at the time they did. And, besides, it is not this sequence which is argued to be detrimental to novelty but, taking into account the wording of claim 2: "**DNA comprising** the DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605.", the 42 Mdal plasmid per se, which contains said sequence and which was available and sequencable to the extent needed, before the priority date (see points 16 and 17 supra).

21. The technical part of the argument is based on the fact that the fragment containing the insecticidal gene of the 42 Mdal plasmid sequenced by the Respondents has a different restriction map from that of the 14 Kb Bam1 fragment containing the insecticidal gene of the 42 Mdal plasmid described in document (11). Yet, in his declaration dated 5 March 1997, the author of document (11) states that the restriction map shown in document (11) contains errors and that the plasmid which was sequenced by the Respondents is the one described in document (11). In addition, the mistakes in the restriction map are already drawn attention to, in the document (22) published before the priority date. For these reasons, the Board concludes that the Respondents provided satisfactory evidence that the sequence of the insecticidal gene in the 42 Mdal plasmid is the same as

that shown in Figure 13 of the patent in suit.

22. Finally, the Appellants also drew the Board's attention to the facts that the DNA probe used in document (11) to isolate the insecticidal gene hybridized to insecticidal genes of other Bacillus thurigiensis strains and that there existed a second insecticidal gene in B.t.berliner 1715 (but not on the 42 Mdal plasmid), implying that this cast doubt on the feasibility of identifying the insecticidal gene within the 42 Mdal plasmid. These facts, however, are not relevant because the experiments involving the 42 Mdal plasmid are carried out in such a way that neither the DNA of Bacillus thurigiensis nor the chromosomal DNA of B.t.berliner 1715 are present.

23. The Board, thus, concludes that, as the 42 Mdal plasmid disclosed in document (27) was available, analysable and reproducible at the priority date, said document destroys the novelty of the subject-matter of claim 2 in accordance with the principles laid down in the Enlarged Board decision G 1/92 (see supra). The main request is rejected.

Auxiliary request 1

Claim 2

24. The subject-matter of this claim which is directed to the DNA sequence of Figure 13 from nucleotide position 141 to nucleotide position 3605 finds support on page 14, lines 53 to 54 of the application as filed. The scope of the claim is narrower than that of granted claim 2 as the claimed DNA is restricted to the insecticidal gene per se. The requirements of

Article 123(2)(3) EPC are fulfilled.

25. The only issue at stake is inventive step. The Appellants identified the closest prior art to the subject-matter of claim 2 as being document (1) which describes the use of a B.t.kurstaki DNA probe to localize the insecticidal gene in 32 different strains of Bacillus thurigiensis. Another document of the state of the art (document (11)) discloses that B.t.berliner 1715 contains an insecticidal gene.

26. In accordance with the case law (e.g. T 989/93, of 16 April 1997) of the Boards of appeal, a document serving as the starting point for evaluating the inventive merits of the invention should relate to the same or a similar technical problem or, at least, to the same or a closely related technical field as the patent in suit. Here, for the subject-matter of claim 2, the closest prior art is document (11).

27. The teaching of document (11) is that the insecticidal gene is located on one of the plasmids comprised within B.t.berliner 1715 and that a DNA fragment comprising said gene is expressed in E.coli to produce a protein with insecticidal activity.

28. Starting from the closest prior art, the problem to be solved can be defined as providing and characterising precisely an insecticidal gene of B.t.berliner 1715.

29. The solution provided is to clone the plasmid DNAs contained in B.t.berliner 1715 in E.coli, to select a recombinant clone capable of expressing the insecticidal protein, to locate the gene, it contains, by deletion analysis and to determine its sequence. The

DNA of claim 2 is, thus, obtained which solves the above mentioned problem.

30. Document (11) teaches the starting material for the isolation of the insecticidal gene (plasmid DNA) as well as the method (gene expression) to use to screen the E.coli gene bank (see point 27, supra). There is no evidence on file that the inventors encountered any difficulties in carrying out the cloning process on the basis of this teaching. The sequencing of the gene was done by a well-established method (page 14 of the application as filed). The claimed sequence did not show any unexpected features. The Board, thus, concludes that no inventive step was required to obtain the DNA of claim 2.

31. Auxiliary request I is refused for not fulfilling the requirements of Article 56 EPC.

Auxiliary request II

Article 56 EPC
Claim 2 (claim 3 of the main request)

32. This claim relates in particular to insecticidally active **truncated proteins** which, all parties agree, are not disclosed in the priority document. Thus, its priority date insofar as this embodiment is concerned is the filing date (17 January 1986).

33. The closest prior art is document (9) published in March 1985 which discloses that only part of the insecticidal gene of B.t.berliner 1715 is necessary for insecticidal activity. Deletions are made in said gene and the corresponding constructs are tested in E.coli

for their capacity to express an insecticidally active protein. It is found that the expression of a deleted DNA of a size inferior to that necessary to encode a protein of 100 Kd does not lead to any protein being stably synthesized (Figure 2, pH1). On the contrary, a construct big enough to encode a 100 Kd protein (Figure 2, pH2), expresses this protein and a 65 Kd degradation derivative thereof (i.e with a molecular weight close to that of the insecticidal toxin) and has a toxicity nearly identical to that of the full length insecticidal protoxin (140 Kdal). It is stated at the end of document (9): "Possibly, the 100- or 65 Kda protein or both were responsible for the toxicity of the pH2 clone."

34. The claimed insecticidally active truncated proteins have a molecular weight comprised between 81 Kdal and 68 Kdal. This molecular weight is within the molecular weight range of proteins which, according to document (9), would be expected to be insecticidally active. Inventive step is, thus, denied.

35. The Appellants drew the Board's attention to the fact that the minimum size for a protein to be insecticidal depended on the Bacillus thurigiensis subspecies, it originated from, and, thus, in their opinion, one could not predict the size range of B.t.berliner 1715 truncated proteins which would be compatible with insecticidal activity. However, in view of the teachings of document (9) specifically relating to B.t.berliner 1715, this comparison does not alter the conclusion on inventive step.

36. Auxiliary request II is refused for not fulfiling the requirements of Article 56 EPC.

Auxiliary request III

Article 56 EPC

37. The closest prior art document to the subject-matter of the remaining claim is document (9). As already mentioned in point 33 above, this document teaches that the DNA constructs which lead to the expression of an insecticidal protein **in E.coli** are those which encode proteins of more than 100 Kdal (Figure 2, pH2). A deleted DNA construct which encodes a truncated protein of **less than** 100 Kdal does not lead to the expression of a stable, truncated protein (Figure 2, pH1).

38. The problem to be solved is to isolate constructs which lead to the expression of insecticidal resistance **in plant cells**.

39. The solution is to isolate chimaeric constructs comprising a plant promoter and carrying deleted fragments of the insecticidal gene encoding proteins of **less than** 100 Kdal. The Board is satisfied that this solution solves the above mentioned problem in view of the results obtained in Examples 13.3 and 13.4 of the patent in suit.

40. Prima facie, this solution is unexpected since it is in direct contradiction with the results obtained in document (9) that an insecticidal protein of less than 100 Kdal could not be produced in E.coli by expression of a DNA construct which encoded a truncated protein of less than 100 Kdal (Figure 2, pH1).

41. The Respondents argued that although the claimed

insecticidal genes were deleted in such a way that they encoded truncated proteins of less than 100 Kdal in size, they were nonetheless of a bigger size than the deleted insecticidal gene in pH1 (document (9)) which did not lead to the expression of a stable protein. In their opinion, a comparison between the construct in pH1 and the claimed constructs for the purpose of assessing inventive step was irrelevant. They pointed out to document (5) where it is shown that deleted B.t.kurstaki insecticidal genes of a similar size to that comprised in the claimed B.t.berliner 1715 chimaeric constructs lead to the expression of stable, insecticidally active proteins.

42. The Board, however, notices from document (5) that the expression of B.t.kurstaki deleted insecticidal genes encoding truncated proteins of less than 100 Kdal (58 Kdal-60 Kdal; page 6274, right-hand column) leads to the synthesis of stable, truncated proteins **in E.coli**. Thus, in spite of the homology which exists between the DNAs encoding B.t.berliner 1715 and B.t.kurstaki HD-1 Dipel insecticidal proteins, truncated derivatives of these proteins do not have the same properties in terms of stability **in E.coli**. Therefore, the results obtained with B.t.kurstaki are not indicative of the results one may expect with B.t.berliner 1715.

43. The skilled person being aware of the teachings of document (9) that a B.t.berliner 1715 truncated insecticidal protein of less than 100 Kdal was not obtainable in E.coli by recombinant expression of the corresponding DNA construct would not have had any incentive to try such construct **in plant cells**. Had he/she nonetheless done so, he/she had no reasonable

expectation of success that the constructs would lead to the expression of insecticidally active truncated proteins **in said plant cells**.

44. The argument by the Respondents that the insecticidal effect in plant cells had only been obtained with one fused construct and, therefore, did not justify acknowledging inventive step over the whole scope of the claim is not accepted. The insecticidal effect was also observed in plant cells using short, non-fused constructs (patent in suit, Example 10.8 and document (26), Figure 1). In addition, the respondents have not provided experiments to show failures using other constructs. The Board is satisfied that inventive step over the whole scope of the claim has been met.

44. For these reasons, inventive step is acknowledged.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The requested correction of claim 1 is allowed.

3. The case is remitted to the first instance with the order to maintain the patent on the basis of the third auxiliary request filed on 9 November with a description to be adapted thereto.

The Registrar The Chairwoman

U. Bultmann U. Kinkeldey