Theor. Appl. Genet. 53, 145-149 (1978)

Genetics of the Ergot Fungus *Claviceps purpurea*.

I. Proof of a Monoecious Life Cycle and Segregation Patterns for Mycelial Morphology and Alkaloid Production

K. Esser and P. Tudzynski
Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Bochum (BRD)

Summary. The morphological and physiological evaluation of single ascorspore lines (F_1) obtained from 4 production strains of *Claviceps purpurea* and the subsequent production and evaluation of an F_2 from two of these strains has shown:

1. The breeding system responsible for the sexual cycle of *C. purpurea* is characterized by monoecism and self-compatibility.
2. Heterokaryosis is neither required for the completion of the life cycle nor for alkaloid synthesis.
3. The huge variability in both mycelial morphology and alkaloid spectra makes it evident that all production strains are highly heterogeneous.
4. A correlation between morphology and an alkaloid pattern was not found.
5. Alkaloid production is, however, correlated with distinct genotypes.
6. In some cases an increase in the total alkaloid content and in the formation of specific alkaloids was also found, showing that the genetic control of these metabolites may be accessible.

Taken together these results open up the way to concerted breeding with *Claviceps purpurea* with the object of improving alkaloid production.

Key words: *Claviceps* life cycle – Alkaloid production – Mycelial morphology

Introduction

The alkaloids produced by the plant pathogen *Claviceps purpurea* have been used in the pharmaceutical industry since the second half of the 19th century as the basis for various kinds of drugs. It is known that the formation of the alkaloids is correlated with a specific kind of mycelial growth, known as sclerotial growth, in which the hyphae are swollen and produce very short branches. This type of growth occurs in parasitic as well as in axenic cultures. During parasitic growth infection of the flowers of the host plant (mainly *Secale*) results in the formation of the typical plectenchymatic sclerotia of seed grains. In axenic cultures, after germination of the spores or inoculation with mycelial fragments, normal hyphal growth can develop into sclerotial growth (for references see Martin and Demain 1978; Mantle 1974).

Although conidial spores are produced abundantly by axenic cultures, the sexual cycle can only be completed by passage through a host plant.

In order to obtain alkaloids for industrial use both modes of cultivation are used; production of sclerotia in the field and production of sclerotial mycelium in fermenters (Spalla 1973). Any improvement of yield in total alkaloid content or of one specific alkaloid would need concerted breeding of the strains concerned. This requires as a prerequisite a knowledge of the life cycle and ability to manipulate it (Esser 1977) in order to get controlled genetic recombination and the possibility to combine appropriate genes obtained either by mutation or by selection from natural sources.

As far as we know (see also Mantle 1974) the genetics of the ergot fungus has not received much attention. There are still contradictory reports as to whether the formation of sclerotia depends on infection with one or two different strains.

Bekesy (1956) has reported that some monoascosporic mycelia can complete the whole life cycle after infection but others can not. He assumed that the second category of spores were 'heterothallic'. Furthermore he pointed out that the offspring are far from homogeneous with respect to colour of the sclerotia and alkaloid content and this phenomenon could arise only if the ascospores are heterokaryotic (Bekesy 1973).

Thus, one aim of this work was to find out whether *C. purpurea* is monoecious and self-compatible or whether its

0040-5752/78/0053/0145/$ 1.00

life cycle is controlled by incompatibility.

Another problem to be resolved is whether or not only heterokaryotic mycelia are able to produce alkaloids as proposed by Amici et al. 1967; Spalla 1973. Furthermore it has been observed that after axenic cultures strains loose their infectivity. They can no longer be submitted to sexual reproduction and are, therefore, useless for breeding procedures. This may also be correlated with strain aging or senescence (for details see Esser and Tudzynski 1978).

In order to obtain insight into the causes of these phenomena which are so essential for practical purposes we have started to examine the genetics of *Claviceps purpurea*. In this first paper we report on the possibilities of performing genetic analyses with this fungus under controlled conditions.

Material and Methods

Strains were obtained from Boehringer, Ingelheim, as sclerotia. Their characteristics are compiled in Table 1.

Culture medium according to Boehringer, Ingelheim: 100 g Saccharose, 5 g Peptone, 5 g Asparagine, 1 g KH_2PO_4, 0.5 g $MgSO_4 \times 7H_2O$, 0.1 g $FeSO_4 \times 7H_2O$, 20 g Agar; in 1 l H_2O; pH 5.2.

Culture conditions: mycelia were kept at 26°C, moisture 70%.

Isolation of mycelia from sclerotia and germination of sclerotia according to Corbett et al. (1975).

Isolation of ascospores: ascospores ejaculated from mature stromata were collected on agar plants. Single spores were isolated under a dissecting microscope according to Esser (1976, p. 44). The germination rate was about 90%.

Parasitic culture: single plants of *Secale cereale* (strain) were infected with conidia taken from axenic cultures of single ascospore lines. After infection the heads were covered with gauze bags until the onset of sclerotia formation in order to avoid subsequent infections (performed by Boehringer, Ingelheim).

Analysis of alkaloids was also performed by Boehringer, Ingelheim, by thin-layer chromatography according to McLaughlin et al. (1964).

Results and Discussion

I. Morphological Analysis of the F_1 in Axenic Cultures

Sclerotia from all four strains were successfully germinated. In order to avoid unnecessary heterogeneity, ascospores were isolated from only one sclerotium of each strain. The resulting F_1 mycelia were grown in axenic culture and their phenotypes morphologically described. For example, 4 mycelia from strain 7 are presented in Figure 1.

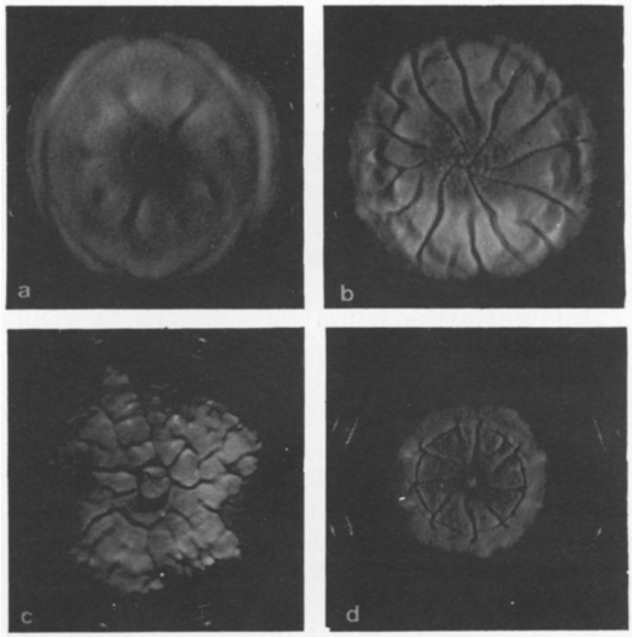

Fig. 1. Mycelia of *Claviceps purpurea* showing the huge versatility of phenotypes obtained from the ascospores of a single sclerotium of strain Nr. 7. a) line 24: mycelium white, lanate; b) line 31: white, center orange, radial grooves; c) line 37: white, irregular grooves, margin partially flat and grey; d) line 29: white, radial grooves, slow growth. The first two phenotypes (a and b) were further analysed (Table 4)

Table 1. Morphological and physiological characteristics of four strains of *Claviceps purpurea*

Strain	Morphological criteria			Production of alkaloids	
	Mycelium		Conidia per cm^2	Main alkaloid	Percentage of total alkaloid content (%)
	Growth rate (mm/d)	Phenotype			
4	2.4	white, lanate	2×10^7	Ergotamin	88
7	1.5	white, lanate, radial grooves	5×10^5	Ergocristin	69
8	1.5	white, flat growth	9×10^6	Ergocristin	67
11	2.3	white, narrow grooves	3×10^7	Ergocornin/ Ergokryptin	63

Table 2. Results of the morphological analysis of F_1 and F_2 ascospore lines. Due to the complexity of morphology the descendents of strains 4 and 11 were not analysed further

Strain	F_1 Generation			F_2 Generation			
	Ascospores germinated	Morphological groups	Ascopore lines forming sclerotia	Formation of stromata	F_1 lines from which spores were isolated	Total number of germinated ascospores	F_1 lines with homogeneity in F_2
4	35	12	–	–	–	–	–
7	48	9	48	36	28	282	17
8	53	11	53	50	33	296	22
11	49	7	49	–	–	–	–

Table 3. Survey of alkaloid production in F_1 sclerotia originating from the cristin strains 7 and 8

Parental strain	Alkaloid segregation in F_1				
	Number of lines assayed	Classification according to main alkaloids			Number of F_1 lines with increase of cristin content
		I Cristin	II Cornin/Kryptin	III Cristin/Cornin Kryptin	
7	48	20	21	7	8
8	53	16	33	3	3

Table 4. Morphological and physiological characterisation of some F_1 lines of strains 7 and 8. As indicated some of the phenotypes may be found in Figure 1

Strain		F_1 Generation					F_2 Generation	
Parental	F_1 line	Morphology	Growth rate (mm/d, 26°C)	Conidia/cm² (26°C)	Alkaloid production		Number of ascospore lines	Morphology
					Group (Table 3)	Main alkaloid (% of total alkaloid content)		
	25	white, lanate	1.7	1.1×10^5	III	67	31	heterogenous
	24	white, lanate	2.0	2.3×10^5	I	77	6	
7	31	white, center orange, radial grooves	2.1	3.0×10^8	II	58	18	homogenous
	38	white, center furrowed radial grooves	2.1	9.4×10^6	III	85	6	
	42	center white, margin grey, radial grooves	2.3	4.1×10^6	I	72	28	
	5	white, lanate, faintly grooved	2.1	5.7×10^8	II	80	6	
	8	white, lanate, faintly grooved	2.4	2.2×10^7	II	72	6	
8	45	white, flat hyphal growth	2.6	3.0×10^6	II	71	35	
	48	white, flat hyphal growth, margin matched	1.8	1.0×10^5	I	78	11	heterogenous

An enormous morphological heterogeneity was observed in mycelial shape, growth rate and conidiation. The huge diversity made it impossible to separate all the strains unequivocally into a distinct number of groups; e.g. for the F_1 of strain 4 as many as 12 groups were needed. Thus the distribution into groups, as shown in Table 2, has to be taken with some reservation.

II. Test of F_1 after Parasitic Culture

Heads of single rye plants were inoculated with conidia obtained from the ascospore lines of strains 7, 8 and 11. In all cases a normal infection with the formation of honeydew and sclerotia was observed (Table 2, column 4). This in itself suggests that *C. purpurea* is monoecious and self-compatible. The F_1 sclerotia were assayed for their alkaloid content with respect to the following points:

1. Do all sclerotia from a single ascospore line have an identical alkaloid spectrum? This was found to be true after analysis of 10 sclerotia from 3 lines derived from strain 7 and two lines derived from strain 8.

2. Is there segregation for alkaloids within the offspring of one parental strain? In order to answer this question we analysed from each ascospore line of strain 7 and 8 a mixture originating from 10-20 sclerotia. As may be seen from the data in Table 3, from two parental strains producing predominantly cristin, a segregation pattern was found. Besides the cristin lines (group I), which were in the minority in both sets of offspring, two new groups with a different alkaloid pattern were found. Group II lines produced cornin and kryptin (so-called toxin lines) and group III lines produced all three alkaloids. Surprisingly, some F_1 lines from both strains showed a quantitatively higher yield in cristin (10-50%).

3. There is no correlation between the mycelial morphology of a line observed in axenic culture and its alkaloid production in parasitic culture. Examples illustrating this statement may be taken from Table 4. Lines 7/24 and 25 belong to the same morphological group but exhibit a different alkaloid spectrum. The reverse is true for lines 7/25 and 7/38 belonging to the same alkaloid group (III) but having different morphologies.

4. Since all sclerotia originated from single ascospore lines it is proved that for alkaloid production no heterokaryosis is required.

III. Morphological Analysis of F_2 in Axenic Culture

Sclerotia of the F_1 ascospore lines were spread out for germination. As shown in Table 2, stromata formed in most cases and ascospores were obtained.

The failure of stroma production in some lines may be due to errors in culture conditions (harvesting time, etc.) but in each case there was at least one germination from the various morphological groups. In this connection it may be significant that sclerotia from those lines, which had a high alkaloid content, germinated poorly or not at all.

The raising of an F_2 is the final proof that *C. purpurea* is monoecious and self-compatible and that no heterokaryosis is needed during the sexual cycle.

From 28 F_1 ascospore lines derived from parental strain 7 and from 33 derived from parental strain 8 ascospores were isolated and their corresponding mycelia evaluated for their morphology.

Since *C. purpurea* was found to be self-fertile, it was expected that all descendents of each F_1 ascospore line would be homogeneous. As may be seen from Table 2 this was the case in the F_2 for more than two thirds of the lines. This again shows that in these cases the whole life cycle took place under homokaryotic conditions.

The heterogeneity found in some lines of F_2 offspring is explained by the fact that during parasitic growth on the rye plant, cross infections caused by migration of very small insects, such as ants, can not be totally excluded.

Another conclusion may be drawn from the data in Table 4: The 3 groups introduced in Table 3 to characterize the alkaloid production are evidently correlated with distinct genotypes since all three groups have been shown to contain homokaryotic strains.

In conclusion there are two points which need to be stressed:

1. The fact that *C. purpurea* is self-fertile and that no heterokaryosis is necessary is of some practical advantage because only one kind of conidia is then required for inoculation in field production. There is no need for double infection or heterokaryons in which the nuclear ratio is hard to balance.

2. Despite the fact that there is not yet any physiological data available concerning alkaloid production in the F_2, the data obtained so far provides the necessary prerequisite for concerted breeding with *C. purpurea*. Furthermore, the information available suggests that it should be possible to understand the genetic control of alkaloid production and to find the genes responsible for specific alkaloids.

Acknowledgement

This work was supported by C.H. Boehringer Sohn, Ingelheim (Germany). We wish to express our gratitude to the staff of the laboratory, especially to Frau H. Hüsemann for their technical assistance.

Literature

Amici, A.M.; Scotti, T.; Spalla, C.; Tognoli, L.: Heterokaryosis and alkaloid production in *Claviceps purpurea*. Appl. Microbiol. **15**, 611-615 (1967)

Bekesy, N.: Über die vegetative und generative Übertragung von Mutterkorneigenschaften. Z. Pflanzenzüchtung **35**, 461-496 (1956)

Bekesy, N.: The inheritance of the alkaloid content of ergot in parasitic and saprophytic cultures. In: Genetics of Industrial Microorganisms. (Eds. Vanek, Z., Hostalek Z., Cudlin, J.) 375-392. Academia Prague 1973

Corbett, K.; Dickerson, A.G.; Mantle, P.G.: Metabolism of the germinating sclerotium of *Claviceps purpurea*. J. gen. Microbiol. **90**, 55-68 (1975)

Esser, K.: Kryptogamen. Berlin-Heidelberg-New York: Springer 1976

Esser, K.: Concerted breeding in fungi and its biotechnological application. Endeavour New Series **1**, 143-148 (1977)

Esser, K.; Tudzynski, P.: Genetic control and expression of senescence in *Podospora anserina*. In: Viruses and Plasmids in Fungi. (Ed. Lemke, P.) New York: M. Dekker (in press)

Mantle, P.G.: Industrial exploitation of ergot fungi. In: The Filamentous Fungi. Vol. 1: Industrial Mycology. (Eds. Smith, J.E.; Berry, D.R.) 281-300. London: Arnold 1974

Martin, J.F.; Demain, A.L.: Fungal development and metabolite fermentation. In: The Filamentous fungi. Vol. 3: Developmental Mycology. (Eds. Smith, J.E.; Berry, D.R.) 426-450. London: Arnold 1978

McLaughlin, J.L.; Ooyan, J.E.; Paul, A.G.: Thin layer chromatography of ergot alkaloids. J. Pharm. Sciences **53**, 306-310 (1964)

Spalla, C.: Genetic problems of production of ergot alkaloids in saprophytic and parasitic conditions. In: Genetics of Industrial Microorganisms. (Eds. Vanek, Z.; Hostalek, Z.; Cudlin, J.) 393-403. Academia Prague 1973

Received May 29, 1978
Communicated by R. Hagemann

Prof.Dr. Karl Esser
Paul Tudzynski
Lehrstuhl f. Allgemeine Botanik
Ruhr-Universität
Postfach 102148
D-463 Bochum