V 薬剤防除

本病防除の有効薬剤を探索し，その使用方法について検討した。

1．室内および圃場検定

薬剤の効果を室内で検定し，そのうちの有効薬剤について圃場の防除効果を検討した。

1）切離葉鞘による検定

a）実験材料および方法

市販の4種類の薬剤を用いた。圃場で慣行栽培したイネの穂孕期の止葉葉鞘を切り取り，10^5または10^7／mlの細菌浮游液を1葉鞘あたり1 ml噴霧接種した。

薬剤処理は，発病を完全に抑制するため接種の前日および翌日の2度切離葉鞘を100倍または1,000倍液に5～6秒間浸漬して行なった。そののちガラス円筒内に入れて湿室とし，20℃に保ち5日後に発病した葉鞘の割合を調査した。

b）実験結果

10^5／mlの菌液を接種した切離葉鞘の発病に対する薬剤の抑制効果はクロラムフェニコール剤が最もすぐれ，100倍，1,000倍いずれでも発病は認められなかった（Fig. 19），次いでストレプトマイシン剤が高かった。

10^7／mlの菌液を接種したときもクロラムフェニコール剤は極めて高い効果を示したが，他の薬剤は殆ど効果が認められなかった。

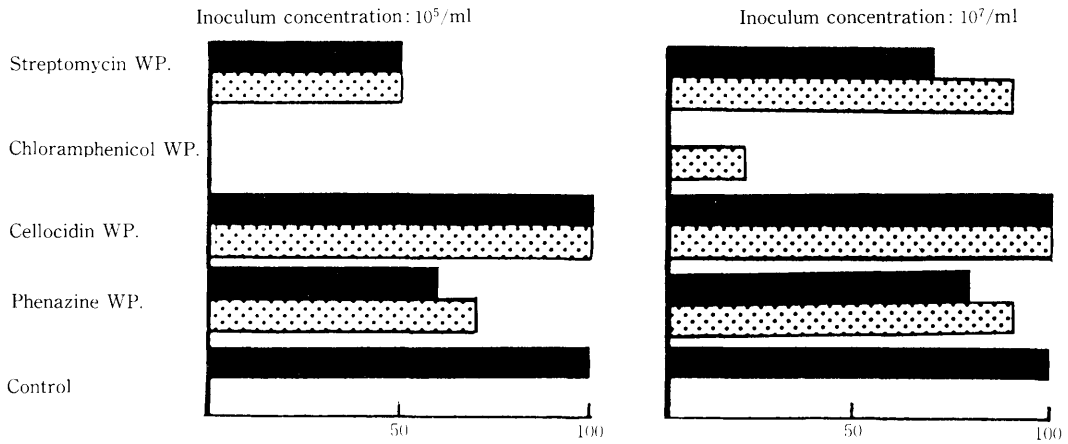

Fig. 19 Control effect of four bactericides applied against disease development on detached flag leaf sheaths that soaked into chemical solutions before and after spraying inoculation. Disease incidence was recorded in 5 days after inoculation.

■ :1/100 dilution, ▨ :1/1,000 dilution

2）ポット栽培イネによる検定

a）実験材料および方法

市販の5種類の薬剤を用いた。ポットに栽培した穂孕期イネの止葉葉鞘に10^7／mlの細菌浮游液を1茎あたり1ml噴霧接種した。

薬剤散布は、発病を完全に抑制するため接種の前日および翌日の2度500倍または1,000倍液を1ポットのイネに20mlずつ散布した。接種7～10日後に病茎率および発病度を調べた。

b）実験結果

発病抑制効果はストレフトマイシン剤、ストレフトマイシン・オキシテトラサイクリン混合剤の2薬剤が高かったが、切離葉鞘による検定ですぐれた効果があったクロラムフェニコール剤は劣った（Table 31）

3）圃場における防除効果

a）実験材料および方法

自然感染による発病に対する薬剤の防除効果を検討した。薬剤は前項のポット栽培イネに対し防除効果のあったストレフトマイシン剤およびストレフトマイシン・オキシテトラサイクリン混合剤を用い、その500倍液を4～5回散布し、成熟期に病株率を30～300株について調査した。

b）実験結果

Table 32に示すようにストレフトマイシン剤およびストレフトマイシン・オキシテトラサイクリン混合剤の発病抑制効果はいずれも高かった。すなわち、両薬剤の500倍液を出穂期の9～15日前から5～6日間隔で5回散布すると無散布に対して発病は著しく少なかった。

Table 31. Control effect of five bactericides against disease development (Pot experiment)[a)]

Bactericide	Dilution times	Disease incidence	Disease severity[b)]	Disease incidence
		1970	1972	1973
Streptomycin WP.	500	−%	35	5%
Streptomycin plus oxytetracycline WP.	500	−	−	27
Chroramphenicol WP.	500	−	81	24
Cellocidin WP.	1,000	72	−	−
Phenazine WP.	1,000	64	−	−
Control	−	67	87	58

a; Chemicals were applied twice before and after spray inoculations
b; Based on assessment of Table 1-(4) category

Table 32. Control effect of two bactericides against disease development under field conditions[a)]

Bactericide	Percentage of affected hills(%)				Reference
Streptomycin WP.	15[b)]	5[c-1)]	1[c-2)]	3[d)]	Streptomycin 20%
Streptomycin plus oxytetracycline WP.	5	7	1	2	Streptomycin 15% plus oxytetracycline 1.5 %
Control	13	21	13	16	

a; Amount of application : 1/500 dilution was sprayed at a rate of 100 l per 10a
b; Date of application: 20, 26 July, 2, 9 August, heading date: 3 August
c; Date of application: 25, 30 July, 5, 11, 16 August, c-1: heading date 9 August , c-2: heading date 7 August
d; Date of application: 28 July, 2, 6, 12, 18 August, heading date 6 August

2．実用的防除

前項で有効であった薬剤のうち，ストレプトマイシン剤は散布により薬剤耐性菌が出現する例が多いが，ストレプトマイシン・オキシテトラサイクリン混合剤は耐性菌が出現し難いとされる(English and Halsema, 1954)。それ故後者について農家圃場で散布濃度，時期および回数を変え，防除効果を検討した。

1）散布濃度

a）実験材料および方法

旭川市の農家圃場で実施した。ストレプトマイシン・オキシテトラサイクリン混合剤の 100倍，500倍および1,000倍液を4～6日間隔で10a当り100ℓ，5回散布した。病株率を 1,600株について所定の日ごとに調査した。実験は1区面積80m²，1区制で行った。

b）実験結果

100倍液，および500倍液を散布したときの病株率は 0.5～1%であり，無散布区の病株率13%に比較すると著しく低く，防除効果があると認められた(Fig. 20)。しかし，1,000倍液散布では劣った。従ってストレプトマイシン・オキシテトラサイクリン混合剤の実用濃度は 500倍液が適当と考えられる。

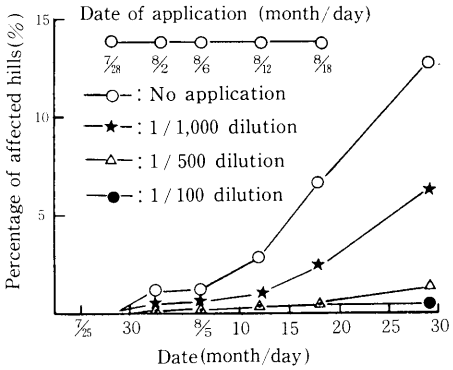

Fig. 20 Control effect of streptomycin plus oxytetracycline WP. applied by three concentrations against disease development under field conditions.(1975)

2）散布時期，回数

a）実験材料および方法

品種「おんねもち」を名寄市の農家圃場で慣行栽培し，ストレプトマイシン・オキシテトラサイクリン混合剤の 500倍液を10a当り 100ℓ，7月20日から8月9日までの間に5日間隔で2回，3

Table 33. Control effect of application date and times of Streptomycin plus oxytetracycline WP. against disease development under field conditions(1976)[a)]

Application date(month/day)					Percentage of affected hills(%)	Disease severity[b)]	Weight of hulled rice	Ratio of hulled rice weight(%)
7／20	25	30	8／4	9	8／13	8／27		
○	○	—	—	—	33	11	223(kg/10a)	97
—	○	○	—	—	25	6	213	93
—	—	○	○	—	17	7	210	91
—	—	—	○	○	24	9	212	93
○	○	○	—	—	9	3	247	107
—	○	○	○	—	19	3	237	103
—	—	○	○	○	41	18	220	96
○	○	○	○	○	4	2	252	110
No application					76	26	230	100

a; Initial occurrence of disease was on 30 July, date of flag leaf emergence on 23 July, heading stage on 7 August

b; Based on assessment of Table 1-(2) category

回あるいは5回散布した。病株率を各区 300株，発病度を 600株について調査し，成熟期に 100株を刈り取り常法により収量調査を行った。

b）実験結果

薬剤の防除効果は散布時期および回数により異なり（Table 33），7月30日の初発病の10日前より5日おきに3回あるいは5回散布したときが最もすぐれ，収量も無散布区に比べ7～10%増加した。しかし，初期の2回散布および後期の3回散布では効果は劣った。すなわち，止葉抽出直前に散布を始め穂孕期まで続けると防除効果がある。なお，出穂期以降低温が長期間続いたため本病は多発し，収量水準は劣った。

以上の結果から，ストレプトマイシン・オキシテトラサイクリン混合剤の 500倍液を止葉抽出直前から5日間隔で3回予防的に散布すれば多発条件下で実用的に有効であると結論される。

3．小　括

葉鞘褐変病菌は病徴を現わすことなく分けつ期のイネ体上および水田周辺のイネ科植物体上に生存し，穂孕期に菌量が増加し感染発病することが先の実験で明らかになった。それ故，穂孕期の菌量の低下をはかることで本病を防除できる可能性があり，薬剤散布による防除を検討した。

切離したイネの葉鞘またはポットに栽培したイネについて薬剤の効果を検定すると，クロラムフェニコール剤，ストレプトマイシン剤，ストレプトマイシン・オキシテトラサイクリン混合剤は発病を明らかに抑制する。しかし，圃場においては後者の2薬剤のみ効果が高い。

この両薬剤は細菌病防除剤として使用されているが，ストレプトマイシン剤を散布すると薬剤耐性菌の出現する例が多く，一方ストレプトマイシン・オキシテトラサイクリン混合剤はその出現を抑制するとされる（English and Halsema, 1954）。それ故，ストレプトマイシン・オキシテトラサイクリン混合剤の散布方法を圃場で検討した結果，発病前の止葉抽出直前から 500倍液を3回予防的に散布する方法が最も発病を抑制するのに有効であった。この防除法の実用性は，その後本病が激発した条件下でも高い防除効果のあることが裏付けられた（秋田，宮島，1976）。

Ⅵ 総 合 考 察

イネ葉鞘褐変病は北日本のイネ栽培地帯で低温年に多発し，玄米の収量と品質の低下により多大な被害をもたらしていた。

イネ葉鞘褐変病菌の種名は1960年代に *Pseudomonas oryzicola*, のち *P. marginalis* とされていた（舟山，平野，1963；舟山，1966）。しかし，本病原細菌について各種性質を検討した結果，Lelliottら（1966）の類別によれば saprophytic fluorescent *pseudomonas* に属しⅤ群に入る。これに対し *P. oryzicola* はⅠ群に該当し，本細菌との相似度も低く87%であった。また，*P. marginalis* の記載（Lelliott et al., 1966；Misaghi and Grogan, 1969；Hildebrand and Schroth, 1971；Köhn, 1973; 太田ら，1976；土屋ら，1979）とはエスクリンの分解，ポリペクテートゲルの液化，硝酸呼吸などの性質に違いが認められた。更に葉鞘褐変病菌の血清学的性質と寄生性は *P. oryzicola* および *P. marginalis* とは異なる。これらのことから本細菌は両種とは別種であると考えられ，他に本細菌に類似する種類は見られない。従って本細菌を新種とし，*Pseudomonas fuscovaginae* sp. nov. と命名した（谷井ら，1976）。その後，国際細菌命名規約（Lapage et al., 1980）の改訂により，本細菌の種名は Approved Lists（Skerman et al., 1980）に掲載されなかった。そこで改めて基準株を6801株（NCPPB 3085,＝PDDCC 5940）に指定し *P. fuscovaginae* sp. nov., nom. rev. と提案した（Miyajima et al., 1983）。

葉鞘褐変病菌を迅速かつ確実に検定，検出する方法として抗血清凝集法，ファージ法およびイネ苗注射法（秋田，沢崎，1972）が有効である。抗血清凝集法は本細菌に対して種特異性が高いので病原菌の検定に利用した。

イネ苗注射法は菌の検出限界が低いが（秋田，沢崎，1972），本細菌の他に *P. avenae, P. glumae* なども検出されるので特異性が劣るとされている（植松，大畑，1977）。しかし，再分離により本細菌との判別を行えば検出精度は高まると考えられる。

葉鞘褐変病菌ファージは3系統あり，いずれも多面体の頭部と尾部を有し，種特異性が高い。このなかでPlファージはファージ放出量が多く，感受性菌も広い地域に分布しているので病原菌の検出に利用しうると考えられる。

ファージを用いた植物病原細菌の検出法はその評価が高い（Katznelson and sutton, 1951；脇本，1955；Okabe and Goto, 1963；Goto and Starr, 1972；小野，1976；Vidaver, 1976）。しかし，ファージの増殖は種々の要因とくに異種細菌が高濃度で混在すると抑制され，検出精度は下るとされる（脇本，1954；後藤，1969；後藤ら，1970；富樫，1976）。

試料中に寄主細菌が7×10/ml 混在しても24時間後にPlファージは増殖する。すなわち，自然環境下の植物生体中の異種細菌数はほぼ $10^{5\sim7}$/gであるので，寄生細菌および異種細菌の菌数とファージ増殖の関係から，植物生体を用いた本法の検出限界は $10^{2\sim3}$/gであると考えられる。従って一般の分離法とともに本法を用い，本細菌の生態研究に利用した。

植物病原細菌の多くは乾燥状態におかれた罹病植物（脇本，玉利，1956；Schuster and Coyne, 1974）あるいは非宿主植物（Ercolani et al., 1974; 伊阪，1974；Gitaitis et al., 1978；Lattorre and Jones, 1979）で越冬するとされる。葉鞘褐変病菌も罹病組織内で塊状になっているため乾燥の下で容易に越冬することができる。また水田畦畔に自生する非宿主のヌカボも本細菌の越冬の場となっていると考えられる。従って本細菌の生存もこれと同じと認められる。

植物病原細菌のなかには宿主植物に病徴を現わすことなく長期間生存するものがあり（Crosse, 1963；Leben et al., 1968；Schneider and Grogan, 1977；Kennedy and Ercolani, 1978），これは居住

型生存形態（resident survival phase）と呼ばれる（Leben, 1965；後藤, 1981）。葉鞘褐変病菌も田面水に垂れ枯死したイネの下葉および健全なイネの茎葉において長期間生存が可能であり，穂孕期近くなると病徴を現わすことなくイネ体上で菌量が高まり，発病はその後に認められる。すなわち，本細菌は分けつ期，止葉期の枯死部で腐生的に生存し，また健全な茎葉上で居住型生存形態をとっていると考えられる。このことは本病が短期間のうちに圃場の全株に発生する例からも裏付けられる。

止葉期から穂孕期の間，イネは止葉葉鞘の重なり合う部分が開く時期以降に発病するが，それ以前のとじている間は発病は認められない。

病原細菌は高い湿度の下で葉上に形成される水膜の中で遊泳することが知られていること(Lucas and Grogan, 1969；Leben et al., 1970)から，葉鞘表面で居住型生存をしている本細菌が高い湿度が3～15時間続くと，葉鞘上の水膜中を遊泳して葉鞘の開いた部分から葉鞘内部に侵入すると説明できる。

次いで葉鞘の病原菌は幼穂組織で増殖して菌量は更に高まり，葉鞘裏面の開いている気孔から侵入する。なお葉鞘表面の気孔からは侵入できない。

植物病原細菌の気孔侵入は正常な形態をした気孔（Daub and Hagedorn, 1979；河本, 木村, 1980）あるいは未熟または退化した異常形態の気孔から侵入するもの（後藤, 1962；田部井, 1967）とがあり，葉鞘裏面の退化した異常形態の気孔から侵入する葉鞘褐変病菌は後者に属する。

本細菌の生活環（Fig. 21）のうち，イネ体における感染経路は，病徴を現わすことなくイネ体に長期間生存し穂孕期以降に止葉葉鞘，穂に感染するとされるイネ籾枯細菌病菌の経路（田部井ら, 1970；十河ら, 1973）に類似すると考えられる。

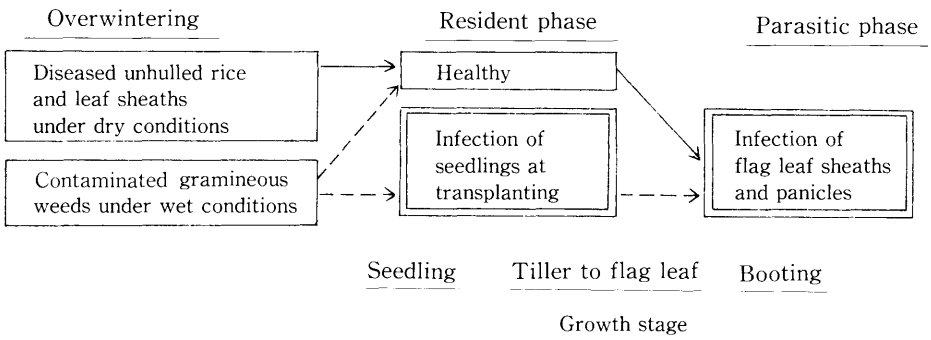

Fig. 21 Life cycle of bacterial sheath brown rot caused by *Pseudomonas fuscovaginae*.

発病には栽培環境よりも気象環境の影響が大きい。

細菌病は一般に多湿の下で多発し，イネ条斑細菌病はRH 83％以上，トマト斑点細菌病はRH 80％以上のとき多発するとされる(Schekhawat and Srivastava, 1972；Yunis et al., 1980)。本病も栗林（1926），秋田，沢崎（1973），千葉ら（1977）が指摘するように冷害年とくに穂孕期が低温多湿のとき多発する傾向がある。穂孕期に高い温度が長時間続くと感染率および発病度が高まる原因の1つは以上の病原菌の活動増加によると考えられる。

また，この穂孕期に低温に遭遇したイネは激しく発病する。

Pseudomonas 属菌のなかには生育適温は高いが低温でも生育し，低温の下で激しく発病するものがあり(Friedman, 1951；Fryda and Otta, 1978；Haas and Rotem, 1976; Young et al., 1977；Schaad et al., 1980)，本病も低温性病害に属する。

発病に及ぼす気温の影響を実験的に検討した結果，低温の下では本細菌の増殖に好適な基質である幼穂が湿度の高い止葉葉鞘内にとどまっている時間が長くなるため，病原菌は幼穂組織で菌量が

高まり，葉鞘は激しく発病する。一方，高温では幼穂が急速に抽出するため，殆ど増殖することができず発病は抑制される。すなわち，本病の発生は，病原菌の生育適温よりもむしろ出穂の速さに左右され，出穂が遅れる低温のとき多く，出穂が早まる高温のとき回避されると考えられる。これらの事実から本病は穂孕期に発生する低温性病害であるとみなされ，この時期に低温多湿に見舞われる機会が多い寒冷地の山間および海岸地帯で多発しやすいと考えられる。

本病の防除は病原菌の生態からみて穂孕期のイネに対する侵入，感染を阻止することで達成されると考えられる。そのためには菌量が高まる止葉期あるいは穂孕期の以前から薬剤を茎葉散布し菌量の増加を抑制することが必要となる。散布時期を検討した結果，菌量が低い止葉抽出直前からの散布により発病の低下と収量並びに品質の向上をもたらしうることが認められた。

北日本の寒冷地帯でイネの安定生産ならびに品質向上をはかるうえに大きな障害となっていた本病は薬剤散布により防除できるが，これに耕種法ならびに抵抗性品種利用なども加えた総合防除法については今後検討する必要がある。

Ⅶ 摘 要

イネ葉鞘褐変病（*Pseudomonas fuscovaginae* Tanii, Miyajima & Akita）の病原細菌の分類・同定，発生生態および防除法に関する研究をとりまとめた。

1．イネ葉鞘褐変病の発生と被害

1）本病には移植後の6月上・中旬に水際部の葉鞘が黒褐色になる苗腐敗と，穂孕期～出穂期に発生する止葉葉鞘および穂の褐変腐敗がある。
2）本病の最初の記録は1926年でその後北海道で常発し，近年の多発年は1962, '64, '65, '66, '69, '70, '75, '76年であり，1976年には東北地方でも発生した。
3）本病が発生すると不稔実粒，玄米の茶米を生ずるので，収量および品質が低下する。

2．病原細菌の分類・同定

1）本細菌は緑色蛍光色素産生の *Pseudomonas* に属する新種であり，*Pseudomonas fuscovaginae* sp. nov. と命名した。
2）本細菌の抗血清は凝集法および寒天ゲル内拡散沈降法によるといずれも本細菌のみと反応し，他の6属24種の細菌とは反応しないので種特異性が高いと考えられる。
3）寄主範囲を明らかにするため24種類の植物に噴霧接種を行った結果，本細菌は5種類のイネ科植物のみに寄生した。

3．病原細菌のバクテリオファージ

1）本細菌に対する3系統のファージが分離されいずれも多面体の頭部を有し，FP1，FP3ファージは収縮性，FP2ファージは非収縮性の尾部をもつ。
2）3系統のファージはいずれも7属34種25病原型の細菌に活性を示さず，本細菌に対し種特異性が高いと認められる。
3）3系統のファージに対する感受性により本細菌は4群に類別され，そのなかでFP1ファージに感受性の菌群は広い地域に分布していた。
4）Plファージの一段増殖は定常期が 240分，平均放出量は 220である。また寄主細菌培養系でのファージ・細菌の増殖関係をみると，細菌数が 10^5 /mlに達してからファージの増殖が始まり 10^9 /mlで定常期になった。
5）異種細菌が混在する場合，Plファージの増殖は抑制されるが，それらの菌数が 10^5 /mlのとき本細菌の検出限界は 7×10 /mlであり，精度が高かった。そこでPlファージを本細菌の生態調査に利用した。

4．病原細菌の生活環

1）室内で乾燥条件の下に保存した被害籾・藁において本細菌は越冬するが，野外に放置した藁では越冬困難と考えられる。また病原菌は被害籾で種子伝染することが認められた。
2）本細菌は田面水に垂れ枯死したイネの下葉，および健全なイネの茎葉上に長期間生存しており，穂孕期近くなると菌量が著しく高くなった。
3）ヌカボその他8種類の植物体上では少なくとも15日または21日まで生存可能であり，圃場に自生するヌカボ，チモシー，オーチャードグラスには6月中・下旬に病徴を現わすことなく生存していると認められたが，その菌量は少ない。

5．病原細菌の侵入と感染

1）分けつ期，止葉期および穂孕期に噴霧接種したイネ，また移植期を変えて生育時期を異にした

圃場のイネでも，全て発病は穂孕期から穂揃期である。このことから感染時期は穂孕期に限られ，幼穂の発育と密接な関係があると考えられる。

2）穂孕期の止葉葉鞘は下位葉鞘よりも感受性が高く，これは葉鞘裏面の気孔の開度が大きいことも関係すると考えられる。

3）本細菌は穂孕期の幼穂では籾の表皮の凹部および内・外穎鉤合部で増殖している。止葉葉鞘では裏面の内表皮表面で増殖し，異常形態の気孔から侵入して柔組織細胞間隙を侵害する。

4）激しく発病した茎は出すくみ穂を生じ，これは感染したみごの組織の壊死により幼穂の抽出が阻害されるためと考えられる。

6．発病環境

1）穂孕期から出穂期が低温のとき多発する傾向があり，低温とくに昼間が低温であるとイネ体における本細菌は活発な増殖を示し，発病は助長されると認められる。

2）穂孕期のイネを高い湿度に保つと感染は高まり，発病は増加すると認められた。

3）移植期，窒素施用量および施肥量を異にしても発病とは一定の関係はなく，これは穂孕期が変るとその時期の気象要因により発病が左右されるためと考えられる。

7．防　　除

1）ストレプトマイシン・オキシテトラサイクリン混合剤の 500倍液を止葉抽出直前から5日間隔で3回散布すると本病は防除できることを明らかにした。

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Studies on bacterial sheath brown rot of rice plant caused by *Pseudomonas fuscovaginae* Tanii, Miyajima & Akita

by

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Summary

Bacterial sheath brown rot, caused by *Pseudomonas fuscovaginae* Tanii, Miyajima & Akita, is a serious foliage disease of rice plant (*Oryza sativa* L.) in northern Japan. In 1918 a disease was discovered on rice plants that was probably caused by this bacterium but the causal pathogen was not found. The disease occurred sporadically to cause severe damage in Hokkaido prefecture. Since then, it has spread gradually to northern Japan and was found in Tohoku prefecture in 1976.

In 1976 160,000 hectares were affected by this disease. The diseased plants yielded less hulled rice and an increased amount of "rusty" rice. Bacterial sheath brown rot is a direct threat to the development of successful rice crops due to its widespread occurrence and severity.

In order to establish practical control measures for the disease, the present studies were undertaken 1 to identify the causal bacterium, 2 to determine the ecology of the disease including the behavior of the bacterium on plants and the environmental conditions favorable for disease development and 3 to determine an effective chemical control method. The results are summarized as follows:

I Symptoms and occurrence

1. Symptoms

The bacterial sheath brown rot usually became noticeable in seedlings after transplanting and at the booting stage of rice plants in the paddy fields.

Seedling rot: Primary symptoms appeared on the lower leaf sheaths at water level as small, water-soaked, greenish-brown spots which then coalesced to elongated streaks or large blotches that were dark brown to greyish brown with no bacterial ooze on the surface (Plate I. 1, 2). The blotches sometimes covered most leaf sheaths. The leaf blades showed streaks along the edges and midribs after the lesions on the leaf sheaths extended to the ligules. Affected hills developed soft rot of the sheaths and wilt of the folded younger blades which pulled out easily and produced a strong unpleasant odor.

Brown rot of flag leaf sheaths and panicles: Initial symptoms appeared on the flag leaf sheaths as

water-soaked, dark green, irregular spots which enlarged to become dark to greyish brown blotches with no definite margins and no bacterial ooze (Plate I. 3, 4. Plate II. 1). Young panicles in diseased sheaths were also attacked. The florets exhibited initially water-soaked brown flecking surrounded by green tissue and finally dark to greyish brown discoloration of complete florets (Plate II. 2). Symptoms on hulled rice in diseased florets ranged from blotches to brown discoloration of whole hulled rice which was named "rusty" rice. Brown necrosis on lower rachis appeared when severe infection occurred on flag leaf sheaths. These lesions were observed on non-emerged panicles (Plate II. 3).

2. Distribution of the disease

Seedling rot was observed in paddy fields in ten districts surveyed. Severe damage to supplemental transplants also occurred exceeding 130,000 hectares in 1969. At the booting stage the disease was observed in 48 districts and occurred frequently in north Sorachi and at the northern limit districts for rice plants at Nayoro and Shibetu cities (Fig. 1).

3. Damage

An experiment was conducted on the damage of diseased plants in paddy field conditions and the effect of growth stage on yields of rice plants inoculated at different stages.

1) Damage of diseased plants in field conditions.

Severe infection of hills increased the percentage of sterility and reduced the weight of hulled rice ; a reduction of 7 to 22 percent was observed in yield when disease severity was moderate, and 22 to 58 percent when it was severe (Table 3).

2) Influence of infection stage on yield losses

Disease severity, percentage of sterility and numbers of "rusty" rice, malformed rice and screening rice increased in plants inoculated at the booting stage but these were much less than those inoculated during the stages of first, middle and full heading time (Table 4). Disease severity and percentage of sterility were highest in plants kept in a phytotron at a day/night temperature of 20/14°C after inoculation at the booting stage than these held at 26/20°C. An increase of 50 to 80 percent was observed in sterility of diseased plants compared with healthy plants when both plants were kept at 20/14°C or 20/20°C (Table 5). On the basis of these results, it may be concluded that bacterial sheath brown rot causes severe losses of yield if the rice plants at the booting stage are infected under low temperature conditions.

II Taxonomy of causal bacterium

A study was made of physiological and serological characteristics, pathogenicity and properties of bacteriophages, and the nomenclature of the organism was examined.

I. Classification and identification of causal bacterium

1) Physiological characteristics

Table 6 lists the 66 cultures tested. They included isolates from flag leaf sheaths, seedlings and unhulled rice collected from Hokkaido and Tohoku prefectures together with *Pseudomonas oryzicola* supplied by Klement, Z. Research Institute for Plant Protection, Budapest, Hungary.

Bacterial cells of the sheath brown rot pathogen were found to be gram negative, nonsporeforming rods with round ends, 0.5 to 0.8 by 2.0 to 3.5μm. Cells occurred singly or in pairs and were motile by means of one to four polar flagella.

On nutrient agar after 4 to 5 days at 28°C colonies were white to light brown, smooth, glistening, raised, translucent, circular and butyrous 3 to 5mm in diameter. A green-fluorescent, diffusible pigment was produced on King's B medium (King et al., 1954). No slime was produced on nutrient agar containing 5 % sucrose.

Metabolism of glucose was oxidative in Hugh and Leifson O-F medium. Catalase and Kovacs.oxidase were positive. Denitrification was negative ; nitrate was not reduced. The MR and VP tests were negative. Lipolysis of margarine was positive. No growth occurred in nutrient broth with 5% NaCl. Litmus milk was peptonized without coagulation and the litmus was reduced. Pits were not produced on polypectate gel at pH 8.5. Starch was hydrolyzed but esculin and arbutin were not. Arginine dihydrolase and ammonia were produced, but cytochrome oxidase, phenylalanine deaminase, urease, 2-ketogluconate, H_2S and indole were not produced. Levan was not produced from sucrose.

No organic growth factors were required. Acid was produced from glucose, arabinose, mannose, trehalose, mannitol but not from maltose, sucrose, raffinose, inulin, salicin, dextrin, adonitol, inositol, dulcitol, sorbitol, or α-methylglucoside. Citrate, malonate, succinate, urate, acetate, β-alanine, L-valine and L-lysine were utilized but not tartrate, hippurate, 2-ketogluconate or polygalacturonic acid.

All cultures were strictly aerobic. The growth temperature was ca. 28°C. No growth occurred at 37°C. Characteristics that varied among strains of the species were as follows: Growth in Cohn's solution, egg yolk reaction, Tween 80 lipolysis, gelatin liquefaction, acid production from xylose, lactose, rhamnose, erythritol.

A hypersensitive reaction was produced when cells were inoculated into tobacco leaves but potato soft rot was not produced (Table 7-1, 2, 3).

On the basis of these results, it may be concluded that the causal bacterium belongs with other arginine dihydrolase and oxidase positive fluorescent pseudomonads. *P. oryzicola* belongs with an oxidase negative group. Consequently, the causal bacterium is a different species from the rice plant pathogens of *P. avenae* and *P. glumae* which are non-fluorescent pseudomonads and from *P. oryzicola* which is oxidase negative. The causal bacterium is most similar to the saprophytic pseudomonads *P. marginalis*, *P. putida* and *P. fluorescens* biotype G. However, there are consistent differences between the sheath brown rot bacterium and *P. marginalis* in the production of β-glucosidase, denitrification, pectate gel liquefaction, potato soft rot and utilization of inositol. *P. putida* differed in gelatin liquefaction, utilization of trehalose and hippurate, starch hydrolysis, and hypersensitive reaction.

P. fluorescens G differed in utilization of arabinose, 2-ketogluconate, inositol, sorbitol and adonitol, and hypersensitivity reaction (Table 7-4).

From the results mentioned above, the causal agent of sheath brown rot of the rice plant was named *Pseudomonas fuscovaginae* sp. nov. 1976. It was however omitted from the Approved Lists of Bacterial Names. Therefore, this name was revived for the same organism in Int. J. Syst. Bacteriol. in accordance with Rule 28a of the *International Code* of *Nomenclature* of *Bacteria* (Miyajima et al. 1983).

The type strain of this species is HK 6801 (=NCPPB 3085 (Harpenden, England), =PDDCC 5940 (Auckland, New Zealand)).

2) Serological characteristics

Agglutination reactions: Both slide and tube agglutination tests were carried out with unheated whole antigens and with an antiserum prepared against whole antigen of *P. fuscovaginae* 6801 isolate. The antiserum diluted 1:100 agglutinated all isolates tested of *P. fuscovaginae* but not any of 40 other nomenspecies including phytopathogenic pseudomonads or unidentified saprophytes isolated from rice plants (Tables 8, 9).

Ouchterlony gel-diffusion reactions: An agar gel-diffusion test was conducted with whole and heated antigen for 1 hour at 100°C according to the Ouchterlony method. In the test using whole antigen, *P. fuscovaginae* antiserum produced four narrow precipitation bands with all isolates of *P. fuscovaginae* but also one to four precipitation bands with nine other species of pseudomonads including eight pathovars. Using heated antigens, *P. fuscovaginae* antiserum formed a heavy precipitation band with this organism but not with 40 other nomenspecies (Table 10; Plate III, 1 to 3). These results suggested that antigen preparations of *P. fuscovaginae* had a specific antigen as well as several antigens in common with other *Pseudomonas* species.

The common antigen bands were eliminated by heating antigens whereas the specific antigen remained.

It may be said that the species-specific antigenic reaction of *P. fuscovaginae* is evident in agglutination and in agar-gel diffusion tests with heat-stable antigen.

3) Host range

Pathogenicity was demonstrated on *Oryza sativa*, *Hordeum vulgare*, *Triticum aestivum*, *Avena sativa*, *Zea mays*, *Lolium perenne*, *Bromus marginatus*, *Phleum pratense* and *Phalaris arundinacea* but was not apparent on *Festuca arundinacea*, two species of *Solanaceae*, six species of *Leguminosae*, *Petroselinum crispum*, *Brassica oleracea*, *Lactuca sativa* or *Citrus limon* (Table 11). On *O. sativa*, water-soaked dark green spots appeared first, then became brown to dark brown blotches on the flag leaf sheath. Infected young panicles showed brown to dark brown discoloration. On the other eight species of *Gramineae*, water-soaked lesions appeared and became greyish white or reddish brown spots on the leaves. It appeared that the host range of *P. fuscovaginae* is limited to gramineous plants.

2. Properties of *Pseudomonas fuscovaginae* bacteriophages

1) Morphology and one-step growth

Fifteen bacteriophages of *Pseudomonas fuscovaginae* isolated from affected flag leaf sheaths, rotted seedlings and diseased unhulled rice in Hokkaido, Japan were classified into three groups based on their host ranges and plaque forms (Table 13; Plate IV, 5 to 7).

The phage particles of all strains were tadpole-shaped consisting of a polyhedral head and a tail. FP1 and FP3 had a head of 60 and 50nm in diameter respectively both with a contractile tail, FP2 had a head of 59nm with a long noncontractile flexuous tail (Table 12; Plate IV, 1 to 4). The one-step growth curve of phage FP1 indicated that the latent period was about 110 min, the rise period was 130 min, and the average burst size was 220 plaque forming units per cell in peptone broth medium at 25°C (Fig. 2).

2) Host range

All three phage strains attacked only the isolates of *P. fuscovaginae* but not any of 34 other species of bacteria including 25 pathovars of pseudomonads. These phages were strain-specific in their infectivity spectra as shown by the fact that 41 *P. fuscovaginae* isolates collected from various localities of northern Japan could be divided into four lysotypes on sensitivity to three strains of phage. Lysotype A including 16 isolates was widely present in six of eight localities tested (Tables 14, 15).

3) The phage-bacteria interaction

In the closed system, P1 phage propagated in peptone broth medium when the bacterial population reached a level of 10^5 cells per ml (Fig. 3). Propagation of P1 phage in the open system mixed with different populations of saprophytes and the host bacterium was tested in peptone broth medium. After 24 hours incubation phage particles of 7×10^2 plaque forming units increased to 3×10^5 in the medium containing the initial bacteria at 7×10 cells per ml mixed with saprophytes at 10^5 cells per ml (Fig. 4).

From the results mentioned above, the phage method was effective in detecting over 10^{2-3} cells of *P. fuscovaginae* per gram fresh weight of plants. Since the population of other saprophytic bacteria on plants was 10^{5-7} cells per gram fresh weight under field conditions, P1 phage was found to be useful in ecological investigations of *P. fuscovaginae*.

III Biology and the disease

The ecological studies of the causal bacteria were conducted using both methods of phage and plating media followed by serological identification. The present work is an attempt to determine the primary infection sources, behavior of the pathogen on gramineous plants, infection of rice plants, epidemiology of disease and environmental factors influencing disease development.

A. Ecology of the causal bacterium

1. Primary infection source

P. fuscovaginae easily overwintered and survived in dry straw and grains kept indoors until next fall but did not survive in straw scattered in paddy fields for two or three months (Tables 16, 17). Infected seeds sown after eight months storage in the laboratory caused disease in rice plants being kept under wet conditions at the booting stage (Table 18). The causal bacteria were also detected in healthy leaves of *Agrostis clavata* var. *nukabo* growing near paddy fields in spring. These results clearly show that

P. fuscovaginae overwinters in dry diseased rice plants and on wild gramineous plants, and that seed transmission of the sheath brown rot is certain.

2. Epiphytic survival of the causal bacterium on rice plants and grasses

1) Multiplication of the causal bacterium on rice plants

The survival ability of *P. fuscovaginae* on rice plants at different growth stages inoculated by spraying was studied in a green house. The pathogen was detected on healthy seedlings until at least 16 days after inoculation of the seeds. The bacterial population increased on rice plants at the booting stage if a bacterial suspension was sprayed at the tillering and flag leaf stages, respectively. Subsequent occurrence of the disease was observed during the booting to flowering periods (Tables 19, 20).

2) Recovery of the causal bacterium from rice plants

The pathogen was detected at population levels of 10^{3-4} cells per gram fresh weight on delayed lower leaves that submerged under irrigation water at the end of June (Table 21). It was also detected at high population on healthy leaf blades and sheaths eight days before diseased plants were visible in the field and then on all healthy plants at two days after the occurrence of disease (Table 22). The results obtained in green house and field conditions show that the causal bacteria survive on healthy rice plants and reach high populations at the booting stage following establishment of the disease. Therefore appearance of the disease is apparently associated with resident populations of *P. fuscovaginae* prior to infection. It may be indicated that *P. fuscovaginae* may go through two phases: a resident and a parasitic phase on rice plants in their life cycle.

3) Survival of the causal bacterium on grasses

The bacteria survived on healthy seedlings of eight species of grasses until for at least 21 days following seed inoculation by soaking them in a bacterial suspension in the laboratory (Table 23). The bacteria were also detected occasionally at a population level not exceeding 10^5 cells per gram fresh weight on symptomless leaves of *Agrostis clavata* var. *nukabo*, *Dactylis glomerata* and *Phleum pratense* near paddy fields on June (Tables 24, 25). These results indicate that the grasses serve as a source of primary inoculum of *P. fuscovaginae*.

3. Infection and host susceptibility

1) Histology of infected plants

As stated previously, the disease generally appeared on the flag leaf sheaths at the booting stage of rice plants. Therefore, a microscopic examination of these infected sheaths was carried out by means of paraffin sections stained with Stoughton's method.

Masses or thin strands of bacterial cells could be seen occasionally on the epidarmal cells of the adaxial side of the intact leaf sheath that had continuously opened stomata and several layers of loose parenchyma beneath it (Plate V. 1, 2). They entered the respiratory cavity through the front cavity and hinter cavity of the opened stomata (Plate V. 3, 4). In the later stages, the bacteria multiplied in the inter-

cellular spaces of the parenchyma beneath the stoma (Plate VI. 2 to 6) and reached the lysigenous aerenchyma. There they multiplied in large masses (Plate VII. 1, 2, 3). However, no bacterial cells were observed in the stomata on the abaxial side of sheath or vascular bundles (Plate VII. 3, 4). Bacterial masses were also observed in the locking parts at the palea and lemma of the hull and on hollow parts of the epidermis of unhulled rice (Plate VII. 5, 6). These results show clearly that the causal bacteria enter the intact flag leaf sheaths through the opened stomata which are present on the adaxial epidermis and progress intercellularly in the parenchma, making it water-soaked in advance.

2) Susceptibility of leaf sheaths at the booting stage of rice plants

Initial symptoms always appeared on flag leaf sheaths but not on lower sheaths at the booting stage of rice plants under field conditions. Therefore, susceptibility of stomata as bacterial entry points in leaf sheaths were studied. The adaxial epidermis of all sheaths were kept wet continuously, and most stomata on these sheaths were open whereas those of the abaxial side of the sheath were closed (Table 26). The stomatal apertures of flag leaf sheaths were greater in number than these of the second and third leaf sheaths (Table 27).

The disease incidence of flag leaf sheath was highest, with less infection in the second sheath and even less in the third and forth sheaths when inoculated under field conditions (Fig. 6). When young infested panicles or absorbent cotton balls were immersed in bacterial suspensions of 10^4 or 10^6 cells per ml and were put in each leaf sheath, severe disease developed on the flag leaf sheath regardless of inoculum dosage while disease development on lower leaf sheaths was slight or absent even at high inoculum concentration in the case of panicle inoculum (Fig. 7). On the other hand, in the case of cotton inoculum more disease developed on the flag leaf sheath at the higher level of inoculum than at the lower level.

From the results mentioned above, stomatal apertures on the adaxial epidermis of flag leaf sheaths were larger than those of the lower sheath and created an avenue of entry for bacteria. Also flag leaf sheaths were more susceptible to infection whereas lower sheaths were resistant. Therefore, causal bacteria initially multiply on panicles and in flag leaf sheath tissues when they reach the adaxial epidermis of these sheaths and subsequently cause disease under favorable environmental conditions.

3) Susceptibility of growth stage to infection

A study was presented to determine the influence of growth stage on susceptibility of rice plants to causal bacteria by different inoculation methods. All the rice plants inoculated by injection at seedlings, tillering, flag leaf and booting stages showed a high percentage of affected plants. On the other hand, spray inoculation resulted in disease only on the plant at the booting stage but not at any one at the other stages (Fig. 9). Furthermore, six growth stages divided arbitrarily by the developmental stage of young panicles comprising the flag leaf stage to the booting stage indicated the difference in susceptibility based on spray inoculation ; Plants became infected at the growth stage when the edge parts of the flag leaf sheath became distant from the other sheaths but not before this stage (Figs 8, 10). These results show that most rice plants at any developmental stage may be infected through wounds but only booting stage plants may be infected when not damaged. Therefore, disease occurrence at the booting stage under field conditions suggests that natural entry points in the epidermis allow invasion by the pathogen in relation to change of developmental growth stage of rice plants.

4) Affected rachis and non-emerged panicle

Affected raches were frequently observed in the non-emerged panicles under field conditions (Fig. 11). When injecting inoculum at the booting stage, an increase of inoculum concentration raised the percentage of non-emerged panicle and of affected rachis (Fig. 12). This suggests that non-emerged panicles are produced by the inhibition of raches elongation which is caused by the bacterial infection.

B. Environmental factors on disease development

Severe outbreaks generally occur in August after periods of cool wet wheather. A study was conducted to determine the influence of climatic conditions and cultural factors on disease development.

1. Climatic conditions influencing disease development

1) Influence of temperature on disease severity and multiplication of bacteria in rice plants

Day temperature rather than night was correlated with disease development and plant heading : Particularly low temperatures at daytime markedly increased the severity of disease and caused late heading while high temperature decreased the severity when the rice plants were inoculated at the booting stage (Fig. 13). Causal bacteria multiplicated markedly in the flag leaf sheath and panicle tissues, and caused severe disease development when inoculated rice plants were kept in a controlled chamber with a day/night temperature of 17/11°C or 23/17°C. There was less multiplication and development at 29/23°C (Fig. 14).

2) Influence of humidity on disease development

Disease incidence was enhanced in 3 hours and reached 100 percent in 15 hours or longer when inoculated rice plants at the booting stage were kept under conditions of 100% relative humidity. Disease severity was also raised consistently with the elapse of time in the same conditions (Fig. 15).

2. Effect of transplanting of seedlings and fertilization on the disease development

Both early transplanting and a decrease in the amount of applied nitrogenous fertilizer caused an earlier initial occurrence of the disease though these factors did not influence disease incidence under field conditions (Figs 16, 17). The amount of nitrogenous, phosphatic or potash fertilizer also showed no relationship to disease severity under artificial inoculation conditions (Fig. 18). These results may be ascribed to the climatic conditions at the susceptible booting stage varying with different cultural methods for rice growing.

IV Chemical control

If effective chemicals can prevent bacteria from infecting the flag leaf sheaths of booting rice plants chemical control of the sheath brown rot may be possible. For this purpose it is essential that chemicals be applied before the flag leaf stage.

Streptomycin and streptomycin plus oxytetracycline proved highly successful on detached leaf sheaths and in pot experiments (Fig. 19, Table 31). Sprays of the latter chemical at different concentrations, dates and times were carried out to determine the control effects because this combination has been known to eliminate streptomycin resistant strains in many bacterial diseases. Three or five sprays at 1/500 dilution applied at five day intervals from 10 days before the initial outbreak showed its effectiveness while two sprays at an early period or later three sprays did not give control (Table 33). It may be concluded from these results that preventative applications of streptomycin plus oxytetracycline mixture (15% plus 1.5%) can be used for agricultural purposes even under severe epidemics conditions.