Proceedings of the New Zealand Grassland Association, 47: 115-120 (1986)

INSECT RESISTANCE, ANIMAL TOXICITY AND ENDOPHYTE-INFECTED GRASS

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Abstract

Endophyte infection is widespread in grasses and in many instances is associated with insect resistance. This resistance appears to be due to chemical components produced in the infected grasses. Ryegrass, infected with Acremonium *loliae* endophyte contains peramine, a feeding deterrent to stem weevil, *Listronotus* bonariensis. Other examples of endophyte-associated insect resistance are discussed and the mechanisms of this insect resistance are briefly compared with those mechanisms implicated in producing animal toxicity problems.

Keywords: endophyte, insect resistance, ryegrass, tall fescue, insect feeding deterrents, animal toxins, alkaloids.

INTRODUCTION

Interest in endophytic fungi infecting grasses dates back to 1898 (Sampson 1933) while endophytes in New Zealand grasses were first reported in 1940 (Neill 1940, 1941). Many different grass species are now known to be infected, each with different endophytes (Clay et. *al.* 1985). Recent research indicates that endophytic infection in grasses is associated with both insect resistance and animal toxicity problems. This paper reviews the current knowledge on endophyte-linked insect resistance and compares the mechanisms of insect resistance with those producing animal toxicity.

INSECTS

Goldson (1979) first found seedlings of perennial ryegrass resistant to Argentine stem weevil. Field resistance was later demonstrated by Kain *et al.* (1982a,b) who recorded less adult feeding and fewer eggs and larvae. on resistant plants. The mechanism of this resistance was clarified when it was shown that the endophyte, *A. loliae,* was present in resistant ryegrass (Prestidge *et* al. 1982). Reduced adult feeding, oviposition and larval development have all since been linked to the presence of endophyte (Prestidge et *al.* 1982, Gaynur and Hunt 1983, Barker et **al.** 1984a,b).

Insect resistance from grass-endophyte associations has now been observed for some eight other insect species (Table 1) but not all such associations lead to insect resistance (Table 2). In some cases insects appear to prefer grasses infected with endophyte. This is true of stem weevil on ryegrass infected with a Gliocladium-like endophyte (Gaynor *et al.* 1983) and cereal aphid on tall fescue infected with a *Phialophthora*-like endophyte (Latch et al. 1985). Resistance can be species specific. Infected tall fescue is resistant to fall armyworm *(Spodoptera jrugiperda)* but is not resistant to southern armyworm *(Spodoptera eridonia)* (Johnson et *al.* 1985).

MECHANISMS OF INSECT RESISTANCE

Studies on the effect of resistant grasses on insect development implicate both feeding deterrence and antibiosis as resistance mechanisms. Reduced feeding of adult stem weevil on infected leaves of ryegrass and tall fescue suggests the presence of a feeding deterrent (Gaynor *et al.* 1983, Gaynor and Hunt 1983, Barker *et al. 1983,* 1984a). Using a choise bioassay based on plant extracts incorported into artificial diets, Rowan and Gaynor (1985) showed that infected ryegrass contains a feeding deterrent. This deterrent, named peramine ($C_{13}H_{17}N_3O_2$), has been isolated and deters adult weevil feeding at Ippm, a concentration comparable to that found in A.

TABLE 1: Insect Resistance in Grasses Infected with Endophyte.

Grasses	Endopyte	Insect: Common Name	Insect: Scientific Name	Reference
Lolium perenne	Acremonium *loliae*	Stem weevil	*Listronotus* bonoriensis	Prestidge et al. 1982
		Sod webworm	*Crambus* spp.	Funk et al. 1983
		Bluegrass billbug	*Sphenophorus paruulus*	Ahmad & Funk 1983
		Fall armyworm	Spodoptero *frugiperda*	Clay et *al.* 1985
		Pasture mealybug	Pseudantonina *poae*	Pearson (pers. comm.)
		Corn leaf aphid	Rhopalosiphum *maidis*	Johnson et *al.* 1985
Festuca arundinacea	Acremonium coenophiolum (*Epichloe typhina*)	Stem weevil	*Listronotus* bonariensis	Barker *et al.* 1983
		Cereal aphid	*Rhopalosiphum padi*	Latch *et* cl. 1985
		Corn leaf aphid	*Rhopalosiphum maidis*	Johnson *et* al. 1985
		Fall armyworm	Spodoptero *frugiperda*	Clay et *al.* 1985
		Greenbug	*Schizaphis graminum*	Siegel et al. 1985
		Large milkweed bug	*Oncopeltus fasciatus*	Siegel et al. 1985
Cyperus *virens*	*Balansia* cyperii	Fall armyworm	Spodoptero frugiperdo	Clay *et al. 1985*
Cyperus pseudouegetus	*Balansia cyperii*	Fall armyworm	Spodoptera frugiperdo	Clay et of. 1985
Paspalum dilatatum	*Myriogenospora atramentrosa*	Fall armyworm	*Spodoptera* frugiperdo	*Clay et al.* 1985
Stipa leucotricha	*Atkinsonella* hypoxylon	Fall armyworm	*Spodoptera* frugiperda	Clay et al. 1985
Cenchrus echinatas	*Balansia obtecta*	Fall armyworm	Spodoptero frugiperda	Clay et *al.* 1985

TABLE 2: Insects Not Affected by Grasses Infected With Endophyte.

Grasses	Endophyte	Insect: Common name	Insect: Scientific name	Reference
Lolium perenne	*Acremonium loliae*	Cereal aphid	*Rhopalosiphum padi*	Latch *et* al. 1985
		Rose-grain aphid	*Metopholophium dirhodum*	Latch et *al.* 1985
		Strawberry aphid	Sitobion *gragariae*	Latch et al. 1985
		English grain aphid	Sitobion *avenae*	Johnson et al. 1985
	Gliocladium-like	Stem weevil	*Listronotus* bonariensis	Gaynor *et al.* 1983
Festuca arundinaceae	*Acremonium coenophialum*	Tobacco hornworm	*Manduca sexta*	Johnson et *al.* 1985
		Tobacco budworm	*Heliothis virescens*	Johnson et al. 1985
		Southern armyworm	*Spodoptero* eridonia	Johnson et *al.* 1985
		English grain aphid	*Sitobion avenae*	Johnson et al. 1985
	Phialophthora-like	Cereal aphid	*Rhopalosiphum padi*	Latch et al. 1985
Festuca *rubra*	Epichloe cf. *typhina*	Cereal aphid	*Rhopalosiphum padi*	Latch *et al.* 1985

loliae-infected ryegrass. Cultures of A. loliae endophyte have recently been found to deter stem weevil feeding and preliminary results suggest they contain peramine (Rowan and Gaynor, unpublished data). This contrasts with results previously reported (Gaynor et al. 1983, Gaynor and Rowan 1985) and suggests that peramine is a fungal metabolite. Prestidge et *al.* (1985) have also reported the presence of a stem weevil feeding deterrent from cultures of A. *loliae* but no pure material has been isolated by these workers.

Feeding deterrence is also implicated in the resistance of infected tall fescue to cereal aphid and greenbug (Latch et al. 1985, Siegel et al. 1985, Johnson et al. 1985). Both aphids quickly move off infected plants and die if caged on them. The basis of this feeding deterrence must be different from that affecting stem weevil because A. loliae-infected ryegrass is resistant to stem weevil but not cereal aphid or greenbug. Johnson et al. (1985) have shown that cereal aphids and greenbugs are deterred by extracts from infected seed which contain high levels of loline alkaloids. These alkaloids are present in high concentrations in infected tall fescue and in very low concentrations in uninfected tall fescue. They are reported to be absent from perennial ryegrass (Aasen et al. 1969). Although final proof that loline is a feeding deterrent for aphids is lacking, it is probable that two different classes of alkaloids (peramine and loline) are implicated as feeding deterrents.

As well as feeding deterrency, antibiosis and toxicity may be involved in endophyte related resistance. Stem weevil on infected ryegrass show reduced oviposition and larval development (Barker et *al.* 1984a,b). Stem weevil larvae reared on diet contain-containing lolitrem B, a neurotoxin isolated from ryegrass infected with *A. loliae*, exhibit slower development rates and higher mortality. However, lolitrem B is not a feeding deterrent to adult stem weevils (Prestidge and Gallagher 1985). Fall armyworm on infected ryegrass and tall fescue show reduced larval survival, weights and longer larval duration times (Clay *et* al. 1985). Extract from infected tall fescue seed was toxic to the large milkweed bug (Johnson *et* al. 1985). While reduced insect growth and increased mortality may also be due to reduced consumption resulting from the presence of afeedingdeterrent, the results of Prestidge and Gallagher (1985) indicate that antibiosis and/or toxicity are also likely to be involved in resistance.

ANIMAL TOXICITY AND INSECT RESISTANCE

Animal health problems have been reported for livestock grazing endophyte infected pasture. Stock grazing infected ryegrass are susceptible to ryegrass staggers (Fletcher and Harvey 1981) and cattle grazing infected tall fescue can suffer from a complex of disorders generally referred to as fescue toxicity (Bacon et al. 1977). Gallagher et al. (1981) reported the isolation of two potent lolitrem neurotoxins from staggers-producing ryegrass pasture. These lolitrems elicit staggers symptoms in mice and their presence in ryegrass has been linked to A. loliae infection. The structures of lolitrem B ($C_{42}H_{55}NO_7$) and C ($C_{42}H_{57}NO_7$) have been determined (Gallagher *et* al. 1984) and are structurally related to the known aflatrem, penitrem and janthitrem mycotoxins suggesting that they are produced by the endophyte (although this has yet to be proven).

A number of chemical constituents of tall fescue have been associated with fescue toxicity and these have been reviewed by Porter (1983). Butenolide A, ergot alkaloids, loline alkaloids and 4-aminobutyric acid have all been considered as possible causative agents but, largely due to the complexity of the fescue toxicity symptoms, no causative interactions have been proven. The loline alkaloids and 4-aminobutyric acid are present in higher concentrations in endophyte-infected plants but, while loline alkaloids reduce feed intake of rats their effect on cattle is not yet known. Ergot alkaloids can be produced in culture by the endophyte of tall fescue, A. coenophialum, and are suspected of causing gangrene symptoms in cattle, but so far fractionation of tall fescue extracts has not linked these compounds with fescue toxicity. Therefore the only compounds clearly linked to animal toxicity and the presence of endophyte are the lolitrems associated with ryegrass staggers.

DISCUSSION

Endophyte infection is widespread in grasses and in many instances is associated with insect resistance. This resistance appears to be due to chemical components present in the infected plants. Tall fescue infected with A. coenophialum contains loline alkaloids which have been implicated in both insect resistanceand fescue toxicity but are probably not the only factors involved.

Ryegrass infected with A. *loliae* endophyte contains at least two substances which adversely affect insects. Peramine is a feeding deterrent to adult stem weevils and appears to be very important in reducing the number of adult weevils and, hence, the number of eggs and larvae present on infected plants. Our preliminary results suggest that peramine is produced by the fungus. If this is confirmed then it would be necessary for endophyte to be present for resistance to occur. Lolitrem B is also present in infected ryegrass and appears to be toxic to stem weevil larvae. Lolitrem B differs chemically from peramine and is not a feeding deterrent to adult weevils. Therefore lolitrem B is probably less important than peramine in determining the resistance of infected ryegrass to stem weevil. Although lolitrem B is similar in structure to other fungal metabolites, there is no proof that it is produced by the fungus. In addition, the lolitrems are associated with causing ryegrass staggers whereas peramine has not been linked to any animal health problems.

The prospect of producing a ryegrass resistant to stem weevil without the undesirable side effect of ryegrass staggers depends on the production of infected ryegrass which contain peramine but not the lolitrems. If peramine and the lolitrems are both fungal metabolites then the next step will be the development of endophytic fungi that produce peramine but not the lolitrems.

Current research shows that the association of grass, endophyte, insects and livestock is very complex. The full extent of these interrelationships and their impact on agriculture remains to be determined.

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