acid and was measured in triplicated samples by the saturation binding method of Brown et al.[8]. Results were expressed as pmoles of cyclic AMP accumulated per mg of MBH protein per 10 min. Statistical analysis of results was carried out by Student's t-test or by analysis of variance followed by Scheffé's test.

Results. The effect of Px on MBH cyclic AMP is shown in figure 1. Pineal ablation increased MBH cyclic AMP accumulation significantly, by 98 and 33%, 3 and 7 days after surgery, respectively. 24 h after Gx an augmented cyclic AMP accumulation was observed in rat MBH (fig. 2). Gx and Px had additive effects, as revealed by the increased MBH cyclic AMP accumulation in rats subjected to Gx 3 days after Px. In agreement with the results of figure 1, Px alone increased MBH cyclic AMP accumulation by 52% 3 days after surgery (fig. 2).

Discussion. Norepinephrine increases cyclic AMP synthesis in rat hypothalamic and cortical slices by interacting with both α- and β-adrenoceptors, the α-adrenergic response requiring the synthesis of prostaglandin E_2[9]. After Gx some of the hypothalamic noradrenergic terminals undergo degeneration, as is revealed by the decreased transmitter uptake[10] and content[11] in MBH or median eminence of Gx rats. Therefore, a possible explanation for the enhanced MBH cyclic AMP accumulation 24 h after Gx (fig. 2) could be a direct effect of the norepinephrine released from degenerating peripheral nerve terminals in situ, known to occur within the first 24 h after nerve section[12]; however, further experiments employing adrenergic blocking agents are needed before a definitive conclusion is reached in this respect. A link between Gx and α-adrenergic mechanisms in MBH is suggested by the increase in α-adrenoceptor sites and α-adrenergic responses in MBH of rats killed 7 days after Gx[10].

There is good agreement between our prior observations on the melatonin-induced cyclic AMP decrease of rat MBH explants[5], and the fact that the abolition by Px of the main melatonin source in the body augmented cyclic AMP accumulation in MBH of rats (fig. 1). Within 16-24 h after Gx, and as a consequence of degeneration of peripheral sympathetic nerve endings in the vicinity of the pinealocytes, melatonin synthesis increases several-fold in rats[12]. Since melatonin decreases MBH cyclic AMP, the additive effect of Px on Gx stimulation of cyclic AMP synthesis is best explained in terms of the removal of a negative influence on the hypothalamic parameter examined. However, the inhibitory influence exerted by the pineal gland and presumably melatonin, is not strong enough to overcome the stimulation of MBH cyclic AMP synthesis produced by the norepinephrine release in situ from degenerating nerve varicosities.

Melatonin depresses in vitro the spontaneous and norepinephrine-induced release of prostaglandin E_2 by rat MBH[13]; at the same physiological concentrations, melatonin also inhibits cyclic AMP accumulation by hypothalamic explants[5]. Since the α-adrenoceptor - mediated effect of norepinephrine on MBH cyclic AMP requires prostaglandin E_2 synthesis[9], the possible involvement of these mechanisms in pinea-mediated influence on cyclic nucleotide levels should be considered.

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Association of the degree of methylation of intercellular pectin with plant resistance to aphids and with induction of aphid biotypes

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Summary. Increased methylation of middle lamellar pectin in plants hinders aphids in penetrating host-plant tissue. In sorghum, a new aphid biotype has overcome this host-plant barrier by having increased pectin methylesterase activity. Results suggest that resistance in crop-plants against sap-feeding insects may possibly be manipulated by altering middle lamellar chemistry either through breeding or use of certain plant-growth regulators.

This paper discusses how resistance of sorghum to an aphid-pest is associated with the structure of middle lamellar pectin in the plant. This material plays a major role in mediating the penetration by stylets of sap-feeding insects through host-plant tissues. The manipulation of the nature of plant intercellular pectins, either through plant-breeding or by application of certain plant-growth regulators, could result in improved resistance in crops against pests whose feeding sites are in localized, internal tissues of plants.

Host-plant resistance (HPR) to insects is frequently ascribed to the presence of secondary plant metabolites[1]. In earlier studies on HPR in sorghum[2] towards the aphid,

Schizaphis graminum (Rondani) (biotype C), p-hydroxybenzaldehyde and dhurrin were identified as feeding deterrents. The cyanohydrin glycoside, dhurrin, is located in vacuoles of epidermal protoplasts and enzymes in the plant responsible for its catabolism and subsequent release of hydrogen cyanide and p-hydroxybenzaldehyde are located in the mesophyll parenchyma[3-6].

However, the mouthparts of biotype C of *S. graminum* penetrate sorghum tissue intercellularly, while proceeding towards the phloem[7], thus avoiding the vacuolar phenolics. Moreover, because of potential autotoxicity, it is unlikely that phenolics are translocated in the phloem at concentrations sufficient to deter aphid-feeding (ED_{50} in synthetic diets to deter aphid-feeding by dhurrin = 0.16% and by p-hydroxybenzaldehyde = 0.13%)[2]. Similar arguments apply to the proposed feeding-deterrent role of Dimboa[8] and other phenolics[9] in wheat against *S. graminum* and other sap-feeding insects which probe intercellularly.

Electronic monitoring of aphid-probing shows that biotype C ingests from the phloem of susceptible sorghum varieties significantly longer than from resistant varieties[7]. Furthermore, the rates of growth and reproduction of the aphid are strongly correlated ($r > 0.90$, $p < 0.05$) with duration of phloem ingestion.

In 1980 a new biotype, E, of *S. graminum* was discovered which feeds and reproduces on sorghum and wheat resistant to biotype C[10,11]. Biotype E ingests from the phloem of a biotype C-resistant variety of sorghum, IS 809, significantly longer than biotype C[12]. But, this longer duration of phloem ingestion by biotype E on IS 809 is mostly a result of its ability to reach the phloem of IS 809 more rapidly than biotype C (table 1). These findings indicate that resistance of IS 809 to biotype C is associated with the relative difficulty this biotype has in attaining the phloem. Histochemical[7] and NMR studies show there is no difference between biotype C-susceptible and resistant sorghum varieties in the amount and structure of lignin in cells surrounding the vascular bundle. Hence, there is not a lignin barrier which differentially impedes aphid-stylet penetration.

It is now proposed that the structural nature of pectin, a biopolymer which functions in plants as an intercellular cement, affects the rate of aphid stylet penetration towards the phloem. Others[13-15] have recognized that aphids, which probe intercellularly, possess a salivary pectinase. Accordingly, it has been found that biotypes C and E of *S. graminum* possess pectinase activity. The extractable pectin content of sorghum varieties susceptible (BOK 8) and resistant (IS 809) to biotype C are nearly equal (table 1). Hence, the difference in the rate of stylet penetration between the 2 biotypes cannot be attributed to a quantitative difference in intercellular pectin between these varieties. However, the methyl ester content of the pectin from the susceptible BOK 8 was only half that of the resistant IS 809 (table 1). The enzyme preparation from biotype E depolymerized pectin isolated from IS 809 twice as rapidly as the enzyme preparation from biotype C, indicating that methylated pectins are broken down more rapidly by the pectinases found in biotype E (table 2). The natural depolymerization of pectin can occur through either a variety of hydrolases (polygalacturonases) and/or by a β-eliminative mechanism (pectin lyases). Neither biotype of *S. graminum* has pectin lyase activity and the polygalacturonase activity is similar for both biotypes (table 2). However, the pectin methylesterase activity of biotype E is twice that of biotype C (table 2). Thus, the more rapid removal of the methyl ester groups of pectin by biotype E increases the rate of depolymerization of the polysaccharide chain. Hence, biotype E has overcome the higher methyl ester content of IS 809 pectin by having greater pectin methylesterase activity than biotype C. The hydrolytic activity of pectinases from the pea aphid, *Acyrthosiphon pisum* (Harris), is also reduced with increased methylation of pectin.

The widespead distribution of pectinases in sap-feeding insects[13-18] suggests that pectins are important components in the interaction between plants and these insects. Altering the structure of intercellular pectin, or other chemical constituents of the middle lamella (e.g., pectinase inhibitors, hemicellulose and/or protopectin) could reduce the effectiveness of insect pectinases. This may have important implications for HPR. The nature of the middle lamella in crop-plants may be manipulated through plant breeding or by application of plant-growth regulators (e.g., ethylene, CCC)[19-21]. Furthermore, because such a basis of HPR does not entail elevated levels of allelochemicals there may be no potential for incompatibility with biological control[22]. However, induction of aphid biotypes might be expected.

Table 1. Pectin properties of, and aphid-probing behavior on sorghum varieties resistant and susceptible to biotype C of *S. graminum*

	Susceptible BOK 8	Resistant IS 809
Time to first contact with phloem (min)* for biotype C	113.9a	201.4b
Time to first contact with phloem (min)* for biotype E	84.7a	97.3a
Pectin content**	3.0%	3.5%
Pectin methyl ester content***	8.3%	16.2%

* Means within a row followed by different letters are significantly different ($p < 0.05$). ** Based on defatted, dried plant material. *** Based on percent of carboxyl groups which are methylated.

Table 2. Specific activities of enzyme preparations from biotypes of *Schizaphis graminum* (units $\times 10^{-3} \times$ mg protein^{-1})

	Biotype C	Biotype E
Hydrolysis rate of isolated IS 809 pectin*	1.08	2.32
Pectin lyase	not present	not present
Polygalacturonase*	3.5	3.8
Pectin methylesterase**	65.0	130.0

* One unit of activity is equivalent to the liberation of 1 µmole of α-galacturonic acid (or production of equivalent reducing end-group) $\times$ min^{-1}. ** One unit of activity is equivalent to the liberation of 1 µmole of methanol $\times$ min^{-1} (70% methylated pectin used in assay).

Experimental. Preparation of and ^{13}C-NMR spectral analysis of lignins from resistant and susceptible varieties of sorghum followed published methods[23]. The ^{13}C-NMR spectra of the lignins in DMSO-d_6 were observed at 25.0349 MHz with 1.0 sec pulses (16 µsec in width). 130,000 scans were accumulated at a spectral width of 10 kHz.

Pectinase activity was assayed viscometrically[24]. The rate in percent decrease in viscosity (% $\Delta\eta \times$ min$^1 \times$ mg protein^{-1}; viz. depolymerization) of 1% solutions was: polygalacturonic acid (0% methyl ester) = 0.622; Sigma pectin (9.7% methyl ester) = 0.137; Sunkist "rapid-set" pectin ($\sim$ 70% methyl ester) = 0.068.

Enzyme assays were conducted at 32 °C, pH 7.5 (25 mM Pi) using semipurified protein; the supernatant after centrifugation (50,000 $\times$ g, 1 h, 4 °C) of fractions collected from gel filtration (Sephadex G-25) of defatted tissue homogenates (at 4 °C) of 1 g freeze-dried aphids. Blank assays used protein vide supra denatured by boiling 15 min. Protein was quantified by the method of Bradford[25]. Enzyme assays were those of Albersheim[26] for pectin lyase (using 70%, 9.7% and 0% methyl ester) Kertesz[27] for pectin methylesterase, and Smith and Stokes[28] for polygalacturonase (using polygalacturonic acid).

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Reduced carbonic anhydrase and Na-K-ATPase activity in gills of salmonids exposed to aluminium-containing acid water[1]

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Summary. Exposure of young specimens of *S. salar* and *S. gairdneri* to aluminium concentrations of 200 µg/l in water at pH 5 induced reductions of 25–40% in the activity of carbonic anhydrase and Na-K-ATPase in the gills.

Acidification of lakes and streams caused by acid precipitation is a growing environmental problem. Fish death, especially of salmonids, has been reported from many countries[2,3]. Laboratory experiments have shown that addition of acid to the water will produce physiological disturbances and eventually death in freshwater fishes. The most important changes seem to be a reduction of the plasma concentration of sodium and chloride[2]. Corresponding changes have also been found in fish from acidified streams and lakes. However, in the natural waters fish death occurs at a higher pH than would be expected from results obtained in the laboratory experiments. This difference is assumed to be caused by the increased concentration of aluminium in natural waters due to mobilization from the ground, induced by the precipitated acid[4]. Certain aluminium compounds have been shown to be especially toxic to fish at pH levels of about 5, with a marked reduction of sodium and chloride concentrations in blood plasma[5]. In a search for possible mechanisms causing the death of fish under such conditions we have investigated the activities of carbonic anhydrase and Na-K-ATPase in the gills of the salmonids *S. salar* and *S. gairdneri*, comparing the activity in fish exposed to low pH and aluminium with the activity in unexposed fish. Carbonic anhydrase and Na-K-ATPase are important in the osmotic and acid-base regulation across the gills[6].

Specimens of fish were obtained from local hatcheries[1]. They were exposed to tap water to which had been added

Table 1. Effect of exposure to low pH and aluminium on carbonic anhydrase activity in the gills and concentrations of Na^+ and Cl^- in blood plasma of the salmon *(S. salar)*. Values are given as mean ± SD. The 'enzyme unit' (EU) is defined as the activity necessary to halve the time of the uncatalyzed reaction[7]. Experiments were performed in June 1981 (summer) and January/February 1982 (winter). Figures in parentheses indicate the number of fish studied

	Size of fish		Carbonic anhydrase activity	Plasma concentrations	
	Weight (g)	Length (cm)	(EU/g)	Na^+ (mEq/l)	Cl^- (mEq/l)
Summer:					
Control	35.1 ± 6.6 (10)	16.0 ± 1.2 (10)	1438 ± 320 (12)	143 ± 4 (4)	127 ± 2 (4)
Exposed	27.8 ± 7.9 (5)	15.4 ± 1.4 (5)	881 ± 82 (5)	105 ± 7 (3)	88 ± 3 (4)
Winter:					
Control	22.4 ± 4.0 (8)	13.3 ± 0.8 (8)	1351 ± 211 (8)	142 ± 14 (16)	122 ± 11 (14)
Exposed	24.4 ± 3.6 (6)	14.2 ± 1.6 (6)	1037 ± 156 (6)	130 ± 11 (5)	110 ± 5 (5)

Table 2. Effect of exposure to low pH and aluminium on Na-K-ATPase activity in gills and Na^+ and Cl^- concentrations in plasma of rainbow trout *(S. gairdneri)*. Values are given as mean ± SD. Experiments were performed during January/February 1982 with 9 fish in each group

	Size of fish		Enzyme (µmol P_i (mg protein)$^{-1}$ h^{-1})	Plasma concentrations	
	Weight (g)	Length (cm)		Na^+ (mEq/l)	Cl^- (mEq/l)
Control	56.1 ± 15.8	17.4 ± 1.2	2.06 ± 0.89	165 ± 11	132 ± 4
Exposed	57.7 ± 16.1	17.5 ± 1.5	1.47 ± 0.47	101 ± 26	92 ± 21