eluting from the DE 52 column at 0.3 M NaCl was found to contain a factor which alike calmodulin was retained in the presence of Ca^{++} on troponin I covalently bound to sepharose and which was specifically eluted in the presence of an excess of EGTA[10]. As shown on figure 2 this factor co-migrates with authentic brain calmodulin in SDS polyacrylamide gel electrophoresis.

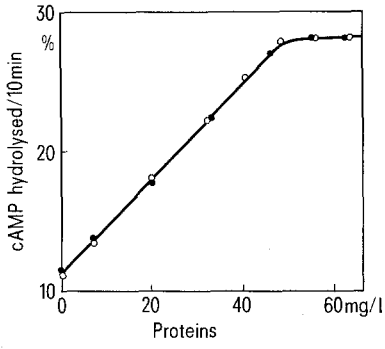

Fig. 3. Dose-response curve for the activation of activator-deficient bovine heart phosphodiesterase by extracts of *Asterias rubens* oocytes. 2 identical oocyte suspensions were used. One received $2 \cdot 10^{-6}$M 1-methyladenine for 15 min. The 2nd served as control. Heat-treated dialyzed extracts were prepared under the same conditions as described in a material and methods, and adjusted to various protein concentrations by dilution with buffer A. The enzyme was assayed with various amounts of the dialyzed heat treated extracts from controls (○) or hormone-treated oocytes (●).

Figure 3 shows that the activation increases rather linearly with the amount of starfish extract to a maximal value. When increasing amounts of starfish calmodulin purified by affinity chromatography were substituted for the dialyzed extracts the same maximal stimulation of the activator-deficient beef heart phosphodiesterase was obtained, which suggested that calmodulin was the only activator of the mammalian enzyme present in the dialyzed starfish extracts. As already reported for the sea urchin oocyte, no sensitivity of starfish oocyte cyclic nucleotide phosphodiesterase towards Ca^{++} could be demonstrated (data not shown). Moreover no change in phosphodiesterase activity occured within oocytes following 1-methyladenine treatment.

To investigate possible changes in calmodulin content or activity following meiosis reinitiation, heat-treated dialyzed extracts were made under strictly identical conditions from starfish oocytes which had or had not been treated with 1-methyladenine for 15 min, a period which is long enough to elicit the appearance of 'maturation-promoting factor' (MPF) within starfish oocyte cytoplasm. As shown in figure 3 doses and responses were found to be strictly superimposable in both cases. This result shows that MPF activity and release from prophase block cannot be correlated with any change in calmodulin content or intrinsic activity (at least towards mammalian phosphodiesterase). It remains possible, however, that there are changes in calmodulin localization within the oocyte like those which have been described in mammalian cells during the time course of mitosis[17], or in the Ca^{++}-calmodulin complex level. These are under investigation.

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Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants

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Summary. Each sex of the cotton leafhopper and the rice brown planthopper communicates with the opposite sex by certain specific sound vibrations which travel through the plant surface and lead to mating. External sounds of certain frequencies, viz., 200 c/sec, generated by a harmonium or an audio-oscillator are picked up from the air by the plants and interrupt the acoustic communication as well as mating of the insects. Notes with harmonics are effective whereas pure notes are ineffective.

Various auchenorrhynchous homopterous insects have been reported to emit acoustic signals[2-8] which, in certain species, travel through the host plant surface and mediate the orientation as well as the approach of the males to the females for mating. The possibility of interrupting the acoustic communication and mating of these insects by external sounds has been investigated in our work on the leafhopper *Amrasca devastans* (Dist), a cotton pest, and the brown planthopper *Nilaparvata lugens* Stål, a rice pest.

The patterns of acoustic signals from the males and females of the brown planthopper have been studied by Ichikawa[8]. The sounds emitted by the cotton leafhopper were recorded

Acoustic communication and mating of the cotton leafhopper *(Amrasca devastans)* and the rice brown planthopper *(Nilaparvata lugens)*[a]

Source of sound	Sound duration (min)	Insect	Fundamental frequency of the note (c/s)	Aerial intensity level[b] (db)	Mean ± SD pick-up of the note (mV)	Mean ± SD Percentage of males reaching/mating with the females[c]
Nil	Nil	*A. devastans*	–	–	–	100[d]
		N. lugens	–	–	–	100[d]
Harmonium	5	*A. devastans*	178	66–68	1.6 ± 5.0[e]	90 ± 10
				72–76	5.0 ± 0.6[e]	23 ± 3
			200	66–68	2.0 ± 0.1[e]	60 ± 20
				72–76	4.5 ± 1.0[e]	10 ± 10
			300	72–76	3.1 ± 1.4[e]	10 ± 10
			400	72–76	3.0 ± 1.3[e]	17 ± 15
			450	72–76	2.0 ± 1.0[e]	17 ± 15
			600	72–76	2.0 ± 1.0[e]	40 ± 17
			1200	72–76	2.0 ± 2.0[e]	70 ± 17
		N. lugens	200	72–76	6.2 ± 1.5[f]	20 ± nil
			300	72–76	0.5 ± 0.2[f]	63 ± 15
			400	72–76	0.7 ± 0.5[f]	57 ± 25
Audio-frequency oscillator type I	5	*A. devastans*	200	80–82	10.0 ± nil[e]	60 ± 20
Audio-frequency oscillator type II	5	*A. devastans*	200	66–67	1.6 ± 0.1[e]	50 ± 17
				73–74	7.0 ± 1.0[e]	Nil
	30	*A. devastans*	200	73–74	7.0 ± 1.0[e]	13 ± 6
	240	*A. devastans*	200 + 300	80–82	7.0 ± 1.0[e]	Nil

[a] A male and a female were released 8 cm apart, each inside a PVC cage, on a plant leaf/shoot. The test sound was generated in the air after the male started 'dancing' (see text). [b] Measured at the level of the test leaf/shoot of the plant. [c] Percentage calculated on the basis of tests with 30 pairs of males and females. [d] Response obtained within the 1st 5 min. [e] Pick-up by the leaves of cotton plants on which *A. devastans* breeds. [f] Pick-up by the shoots of rice plants on which *N. lugens* breeds.

by the same method[8] using 5–7-day-old unmated males and females which show a maximum mating response[9].

A male and a female leafhopper were released on a cotton leaf, and each insect was covered with a removable PVC cage (2 cm in diameter, 1 cm high). A phonogram cartridge (ACOS No. GP91-SC, Cosmocord, UK) had its stylus in contact with the leaf to pick up any surface-borne sounds. A microphone (model ADM-5, Akai, Japan) was placed at the level of the leaf to pick up any aerial sounds. The sounds picked up by each were amplified 1000 times by a preamplifier (type 122, Tektronix Inc., USA), displayed on an oscilloscope (type 502A, Tektronix Inc., USA), recorded on a tape recorder (model 3960A, Hewlett-Packard, USA) and filmed with a kymographic camera (model C-4, Grass Instruments, USA) at 25 cm/sec.

After a male and a female were released on a cotton leaf, generally the male first emitted 'croaking' sounds in units of 2–6 syllables each (figure 1,A). The female then responded with monosyllabic 'cooing' sounds (figure 1,B) which stimulated the male to perform his characteristic 'dancing' movements. After the male's cage was removed, he advanced towards the female and continued to 'dance' on and around her cage. After the female's cage was removed, the male approached and mated with her. Similar behaviour was shown by the brown planthopper on its host (rice) plant. A discontinuity in the surface between the male and the female resulted in the failure of the communication and mating between them. This confirmed that the acoustic signals were received by both sexes from each other through the plant surface.

For interrupting the mating in the pests for their control by external sounds, the foremost requirements would be for the sounds to be generated in the air, to be picked up by the host plants and then to interfere with the acoustic communication between the sexes. For this purpose, sounds from various devices were generated in the air for 5, 30 or 240 min continuously after a male, released about 8 cm from a female on a plant leaf/shoot, started to 'dance'. A decline in the percentage of the males reaching and mating with the females in the presence of the external sounds would reflect their interference with the acoustic communication between the sexes.

Although a number of devices, running motors, vibrators, etc., produced sounds which were picked up by the plants and which interrupted mating in the insects, these sounds were much too noisy and unpleasant to be used for pest management. Hence, sounds from certain musical instruments were tested.

One satisfactory instrument was a harmonium. When the intensity of its various notes in the air at the level of a plant

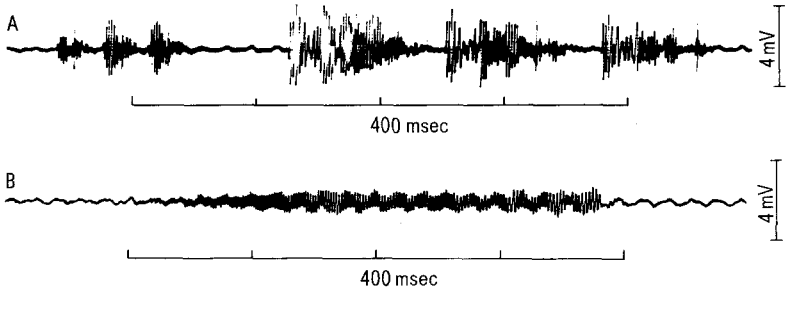

Fig. 1. Oscillograms of the sounds produced by *Amrasca devastans* released on a cotton leaf. *A* male, single 'croak': 3 short syllables followed by 3 long syllables. *B* female, single, monosyllabic, 'cooing' sound. Signal amplitude varies with the distance between the cartridge stylus and the insects on the leaf.

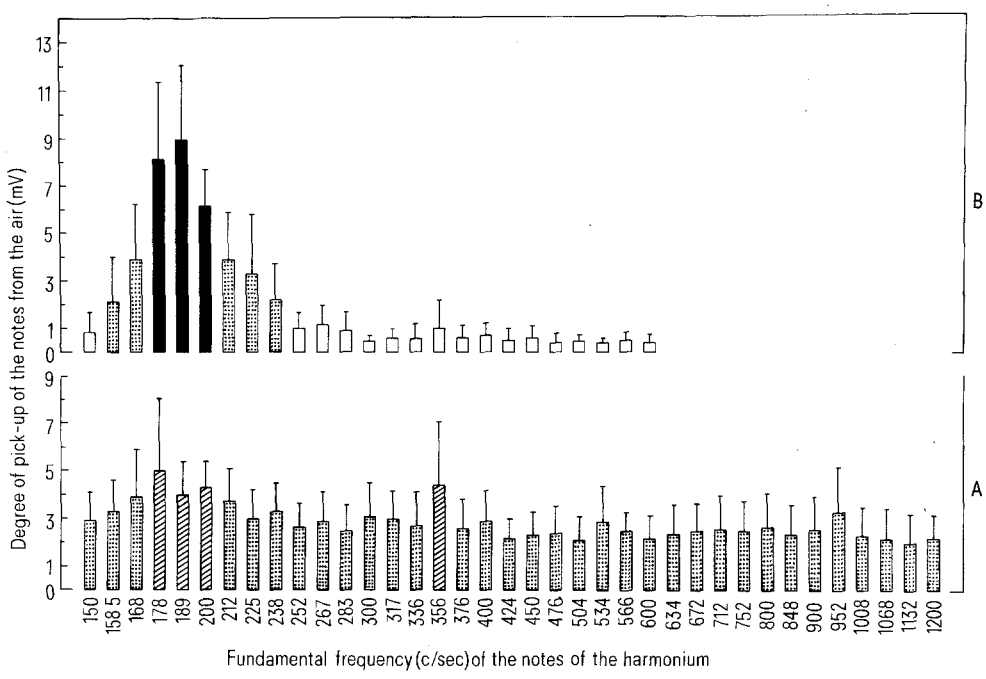

Fig. 2. Degree of pick-up (mean ± SD) of various notes of a harmonium from the air by *A* cotton leaf, and *B* rice shoot. Each note's aerial intensity was maintained almost constant such that, after its pick-up by a microphone directly from the air at the level of the plant leaf/shoot, the electrical signal monitored on the oscilloscope had an amplitude of 20 mV. The degree of pick-up of the note by the plant leaf/shoot was given by the amplitude (mV) of the signal picked up by the crystal cartridge, amplified and monitored on the oscilloscope (as described in the text). The degree of pick-up of the various notes was:
■ very high (≥ 6 mV) ▨ high (≥ 4 < 6 mV)
▦ medium (≥ 2 < 4 mV) □ low (< 2 mV).

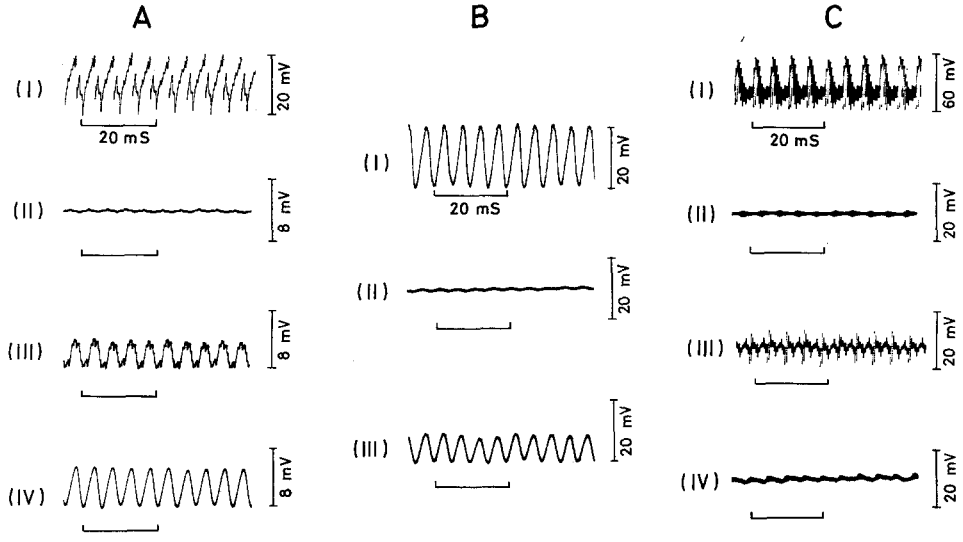

Fig. 3. Oscillograms of the 200 c/sec notes from different sources, picked up directly from the air or through a cotton leaf or a rice shoot. *A* The note from the harmonium picked up by I the microphone from the air, II the cartridge from the air, III the cartridge through a cotton leaf, IV the cartridge through a rice shoot. *B* The note from the type I audio-frequency oscillator picked up by I the microphone from the air, II the cartridge from the air, III the cartridge through a cotton leaf. *C* The note from the type II oscillator picked up by I the microphone from the air, II the cartridge from the air, III the cartridge through a cotton leaf, IV the cartridge through a rice shoot.

leaf/shoot was maintained 72–76 db, equivalent to 20 mV amplitude of the electrical signals displayed on the oscilloscope, the notes were picked up from the air in different degrees by the cotton (figure 2, A) and the rice (figure 2, B) leaf/shoot.

The cotton leaf picked up the harmonium notes with their harmonics (figure 3, A (III)) whereas the rice plant picked up the same notes without harmonics (figure 3, A (IV)).

The acoustic communication and mating of the leafhopper on cotton leaves was interrupted in varying degrees by different notes of the harmonium (table), 200 c/sec and 300 c/sec being most effective. The differences in the interruption by different notes were determined by their frequency, degree of pick-up by the leaves, or both. Thus, 200 c/sec was more effective than 178 c/sec though both the notes were picked up by the cotton leaves almost

equally. 300 c/sec, though picked up by these leaves less than 200 c/sec, was equally effective. With the rise in the frequency above 300 c/sec, the interruption of the communication and mating in the leafhoppers declined until at 1200 c/sec as many as 70% males reached the females to mate with them. A decrease in the sound intensity level of the notes to less than 70 db in the air resulted in a decreased pick-up of the notes by the leaves and, hence, in a decline in the interruption of mating.

The acoustic communication and mating in the rice brown planthopper on rice plants was also interrupted, mostly by 200 c/sec and less by 300 or 400 c/sec (table).

In view of the above, 200 c/sec would be quite a suitable sound frequency for use in pest management programs since it is less disturbing and more effective than other notes at a medium aerial sound intensity level (72–76 db). The next requirement would be to produce the desired note at an almost constant intensity for a long time. For this purpose, 2 types of audio-frequency oscillators were tested: type I (Eastern Electronics, Faridabad, India) generated pure notes (figure 3,B) whereas type II was an electronic tuner (Bina Musical Stores, Delhi, India) which generated various notes with their respective harmonics (figure 3,C).

The 200 c/sec note (pure) from the type I oscillator, although picked up by the cotton leaf (figure 3,B (III)) more than the note from the harmonium, did not interrupt the acoustic communication and mating in the leafhoppers (table), possibly because the note lacked harmonics. However, the same note with harmonics from the type II oscillator, which was picked up equally well by the cotton leaf (figure 3,C (III)) but very little by the rice plant (figure 3,C (IV)), was quite effective in this respect over a period of 5 as well as 30 min (table). A combination of 200 and 300 c/sec was effective up to 4 h. Thereafter, even if the sound was stopped, the interruption of the acoustic communication and mating in the leafhoppers persisted for the next 4 h, only 26.6% males reaching the females for mating during this period.

The present observations thus suggest the possibility of using musical sounds for the control of these insect pests by interrupting their acoustic communication and mating. However, for such an application of sound, it would be necessary to select suitable frequencies and intensities of sound and to program their presentation schedule in such a manner as to minimize noise pollution and maximize the interruption of the acoustic communication.

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Effect of cell synchronization techniques on polyamine content of HeLa cells

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Summary. Synchronized cultures of mitotic HeLa cells were obtained by different protocols and the polyamine content of these cells determined. It was found that the method of synchronization can significantly change the polyamine content of the mitotic cells, and can also alter the time course of polyamine accumulation during the subsequent cell cycle.

There is considerable evidence that polyamines are involved in the maintenance of cell growth[3,4]. Changes in the rate of cell proliferation are always paralleled by changes in polyamine levels[5–8] and it is of great interest, therefore, to examine how polyamine content changes during the fundamental unit of cell proliferation, the cell cycle. To obtain sufficient cells to analyze biochemical parameters during the cell cycle, it is necessary to use a synchronization technique[9]. However, many of the standard techniques are known to produce unbalanced growth[10,11] or cause irreversible damage to the cells[12], and it is therefore important to determine whether such techniques can perturb polyamine content.

Materials and methods. Human HeLa-S3 cells were routinely grown in suspension culture at 37 °C, in Eagle's minimal essential medium[13] supplemented with 5% (v/v) fetal calf serum. Different synchronization protocols were used to synchronize the cells. Protocol 1: medium from subconfluent cultures of cells was aspirated to remove dead cells. The dishes were washed with 5 ml prewarmed medium and then 15 ml prewarmed medium was added. After 2 h the dishes were shaken to dislodge mitotic cells, the medium removed and the cells collected by centrifugation. Protocol 2: 4 million cells were plated out into 140 mm petri dishes and incubated for 12 h at 37 °C. The dishes were shaken and the medium aspirated to remove floating cells. The dishes were washed with 5 ml prewarmed medium, 15 ml prewarmed medium was added and the cells synchronized by incubating the dishes in an atmosphere of 95% N_2O:5% CO_2 under 5×10^5 Nm^{-2} pressure at 37 °C for 3.5 h[14]. The mitotic cells were removed by gently shaking the dishes. Protocol 3: thymidine was added to cells grown in suspension culture to produce a final concentration of 2.5 mM, and left for 19 h. The cells were centrifuged, resuspended in fresh medium without thymidine and seeded into petri dishes. The cells were left for 5 h before a 7.5 h nitrous oxide arrest was commenced. Protocol 4:

Table 1. Polyamine content of mitotic HeLa cells selected by different protocols

Synchronization protocol	Polyamine content (nmoles/million cells)		
	Spermine	Spermidine	Putrescine
1	1.9±0.2	3.7±0.3	0.73±0.1
2	2.0±0.4	4.0±0.4	0.86±0.1
3	1.2±0.1	2.0±0.3	1.40±0.2
4	4.0±0.4	6.0±0.4	ND

Each value is the mean of at least 6 determinations±SEM. ND, not determined. Polyamine content was determined by the dansylation method[16].